THE ANTIDIABETIC FUNCTIONS OF THE PANCREAS

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THE SUCCESSFUL ISOLATION OF THE ANTIDIABETIC HORMONE—INSULIN

J. J. R. MACLEOD

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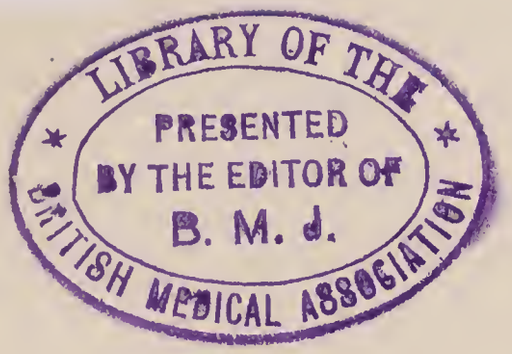

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SUBJECT

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THE SUCCESSFUL ISOLATION OF THE ANTIDIABETIC HORMONE—INSULIN

BY

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1923

To the men who gave service abroad and
at home during the World War,
this book is respectfully
dedicated

PREFACE

This is the second monograph of the Wayne County Medical Society Beaumont Foundation Lectures, the inaugural addresses having been delivered by William G. MacCallum, Professor of Pathology, Johns Hopkins University, in January, 1922, under the subject Inflammation.

It is most appropriate that medical history in Detroit should find two links of such paramount significance as a Beaumont commemoration and the formal detailed announcement of a successful cure for diabetes mellitus. The labors of Beaumont were anatomically confined quite closely to the stomach and its physiology. The work of Professors J. J. R. Macleod and F. G. Banting has been specifically directed to the basic knowledge relating to pancreatic physiology and the therapy of its deficiency disease, diabetes mellitus.

There is added interest for the reader of this monograph since one of the authors has reviewed the results of the experimental investigations which have been made with insulin justifying the recommendation for the use of this remedy in the treatment of diabetes in man. The reviewer, experimenter and discoverer, Professor Banting, gives due credit to Moses Barron, a writer in the journal of *Surgery, Gynecology and Obstetrics* of November, 1920, for suggesting the idea from which this work developed. To Professor Macleod, Dr. Best, Dr. Collip, and the splendid corps of associates at the University of Toronto, he has given credit for aid in the developmental research.

Admiration must be felt for the spirit of cooperation exhibited by the group of workers who have been so successfully led by Professors Macleod and Banting in the accomplishment of one of the outstanding advances in medical therapeutics since the days of Beaumont.

The authors have recorded the historical events in the development of our knowledge of the pancreas, also the function of this organ relating to carbohydrate digestion and metabolism, and have told the story of the discovery and isolation of insulin and its employment in the treatment of diabetes mellitus in a most interesting way.

Permanent and world-wide recognition of this splendid accomplishment in medicine has been made by the 1922 award of the Alfred Nobel prize to Professors Macleod and Banting, thereby specifying their work as the most noteworthy for the year, in the domain of medical science.

With the finest spirit of fairness and professional generosity the recipients divided the award with their coworkers, Drs. C. H. Best and J. B. Collip.

It is most gratifying that we have this historical record prepared in monographic form and issued in the series of Beaumont lectures.

James E. Davis, A.M., M.D.
For the Committee.

January 29, 30, 1923.
William M. Donald, M.D., President,
W. C. M. S.

BEAUMONT LECTURE I.

BY PROFESSOR J. J. R. MACLEOD

January 29, 1923

The Pancreas in Its Relation to Digestion and Metabolism of Carbohydrates—Historical

The first inkling that the pancreas is necessary for the complete utilization of carbohydrates in the animal body dates as far back as 1682. In this year Brunner published a work entitled "Experimenta Nova Circa Pancreas" in which he describes the general behavior of dogs after removal of all of the pancreas save the portion which we now call the tail or splenic end. The description of one of these observations is particularly interesting. Brunner had removed the spleen; and after the animal had recovered from the immediate effects of this operation, he then removed the pancreas with the result, to quote from the translation given in Sir Michael Foster's "History of Physiology," that "it was especially to be seen that the animal made water very frequently and that he was very thirsty, drinking largely of water in proportion to the discharge of urine." Brunner, therefore, discovered that the removal of the pancreas produces the cardinal symptoms of diabetes. In another experiment in which the pancreas alone was removed, Brunner remarks, "I had bought the animal from a butcher and after the operation it also was hungry. It was continually going to its old master's shop and stealing pieces of meat. Indeed, it carried on this game to such an extent that the butcher

came to me and demanded that it should be killed. This, however, I put off doing since I wanted to enjoy for some time longer such a pleasant experience as the animal's condition afforded me.''

The general conclusion which Brunner drew from his observations on depancreatised animals, however, was that they in no way suffered in health and he really did the experiments for the purpose of proving that van Helmont and Sylvius were wrong in ascribing to the pancreatic juice any important rôle in the digestion of foods. He, with his friend Peyer, who you remember discovered lymph nodes in the intestinal mucous membrane, thought that the pancreas was of no importance, but that intestinal digestion was carried out by Brunner's glands and Peyer's patches. The effect of these researches was that for more than a century and a half no one thought it worth while to seek for any function of the pancreas. In the standard work on Physiology by Johannes Müller, published about 1830, the function of the pancreas receives little attention—a few sentences at most—although pages and pages are devoted to the function of the stomach. The reason for this is particularly interesting to the audience that is gathered here this evening because Müller's emphasis of gastric digestion depended on the fact that he had come to know in detail of Beaumont's work on Alexis St. Martin. So much impressed was he with the significance of this work that he apparently regarded gastric digestion as practically the only important part of the digestive process although he also paid some attention to the digestive function of the bile.

The true rôle of the pancreatic juice in digestion was first clearly described in 1856 by the great master of modern experimental physiology, Claude Bernard, and let me remind you of the experiment which attracted Claude Bernard to this field. At the time he was study-

ing the comparative physiology of the digestion of herbivorous and carnivorous animals, and he noted, on examination of the intestine and its mesentery after the animal had been given a meal of fat, that there was a striking difference between herbivorous animals, such as the rabbit, and carnivorous animals, such as the dog. In the former he noted that the lymphatics do not become white from absorbed fat for some considerable distance below the pylorus, whereas in the latter the whiteness starts much higher up. The significance of this observation might have escaped most minds. To Claude Bernard it was a clue to further investigation and he soon found that the cause for the difference depends on the fact that the pancreatic duct in the rabbit enters very low down compared with the position at which it enters in the dog. That led him to investigate the function of the pancreatic juice in connection with the digestion of fat and it was not long before he demonstrated its emulsifying and saponifying powers, and then, with the assistance of his friend, Barreswil, the chemist, he soon after discovered its effect on carbohydrates and proteins. These researches of Claude Bernard on the function of the pancreatic juice were very thorough and comparatively little has since been added to what he discovered. Some details of the method by which protein breaks down have indeed been learned, but we know little more of the effects produced on fats and carbohydrates. Claude Bernard in this as in most of his work cleared up the particular field in which he was working very thoroughly so that he did not apparently leave to others much that was fundamental to discover. But it is not my intention to discuss in these lectures the digestive functions of the pancreas but rather the functions of that gland by which it prevents diabetes.

The Relationship of the Pancreas to Diabetes

In 1788, Cowley, an English physician, first suggested the relationship of the pancreas to diabetes, and it was again referred to by Thomas Bright in 1833. In 1845, the French physician Bouchardat, definitely described a relationship between disease of the pancreas and diabetes, and from that time on confirmatory evidence gradually accumulated in support of this view, but no one ventured to suggest the nature of the relationship. Claude Bernard was naturally interested in this growing conviction that the pancreas is partly responsible for diabetes, and he proceeded to study the effect of its extirpation and of injection of its ducts with paraffin in order to see whether diabetic symptoms might thereby be produced. His results were entirely negative, from which we must conclude, in the light of more modern work, that he did not succeed in entirely extirpating the gland.

Clinical and Experimental Observations

It is interesting in this history to trace the relationship between clinical observations on man and experimental investigations on laboratory animals. The clinician gave the clue, but it was impossible to prove, even by the aid of careful pathological examination, whether or not disease of the pancreas is causally related to diabetes. One investigator would contradict the other in his conclusions and absolute certainty was not forthcoming until it could be shown that experimental extirpation of the gland causes the cardinal symptoms of the disease. I would like to emphasize this because one often fails to realize the exact relation between clinical observation and experimental investigation. The two must go hand in hand. One often thinks of the research man as being inapproachable within the walls of

his college, unwilling to open the locked doors of his laboratory. But this is far from being the case, for the modern laboratory man in the science of medicine must keep his doors open and be ever ready to confer with the clinical observer about his problems so that he may develop his experiments towards the acquisition of knowledge that will be useful at the bedside. I think the history of diabetes shows more clearly than anything else I know of the value of cooperation between experimental and clinical workers.

Others besides Claude Bernard attempted to extirpate the pancreas, but with no success until 1889 when at last, as I suppose most of you are aware, Minkowski and von Mehring succeeded in producing marked diabetes in dogs by complete extirpation of the gland.

About twenty years prior to von Mehring and Minkowski's discovery, Langerhans, an anatomist, had properly described the peculiar collections of cells in the pancreas, now known as the Isles of Langerhans, and Kühne and Lea a few years later had shown that these collections of cells have a very definite and abundant blood supply. Stimulated by the success of Minkowski's work, further investigation of the broadly anatomical features of the pancreas was undertaken, and among the many who contributed to the work, Laguesse and Diamare deserve special mention, for they showed, not only that the islets are distinct and separate from the secreting cells although, according to Laguesse, transformations might occur between islet and acinar tissue. They recommend that close search should be made for lesions of the islet cells in diabetes.

Sir E. Sharpey Schäfer definitely suggested in 1895 that pathological changes in the islets might be responsible for diabetes. Supported by an ever accumulating mass of evidence—anatomical, experimental and clinical —the hypothesis that there is an internal secretion of the

pancreas which controls carbohydrate metabolism grew quickly and was definitely formulated by Lepine, having, however, also been suggested by Minkowski. The hypothesis soon gained strength by the discoveries which came to be made of the internal secretions of the thyroid and suprarenal glands with which you are all familiar. But strong though all this evidence might be, it could not be considered as final until it could be shown that the symptoms of diabetes are removed in a depancreatised animal, or in a diabetic patient, by administration of an extract of the pancreas. This is the essential test upon which such an hypothesis must depend. Up to this stage the evidence was open to question since one could always argue that the reason the pancreas, or the Isles of Langerhans, prevents diabetes is, not because it produces an internal secretion which passes into the blood and acts on metabolism so as to prevent the accumulation of sugar, but because the blood in circulating through the pancreas undergoes some change which removes substances from it which would otherwise cause disturbances of carbohydrate metabolism.

The only test for the hypothesis of an internal secretion of the pancreas is to demonstrate its presence in an extract of the gland by showing that the symptoms of diabetes can be removed by administering the extract to a diabetic animal. Partly to secure this evidence and partly to produce a pancreatic extract that would be useful in the treatment of diabetes a vast amount of work was done, but I will mention only a few of the earlier workers such as Lepine and Hédon in France, and Hale White, Rennie and Fraser in Great Britain. Their researches did not meet with sufficient success to justify the administration of their extracts in the treatment of diabetes. The most successful of all these earlier workers was undoubtedly Zuelzer, a German investigator, who in 1908 succeeded in preparing from the

pressed out juice of adult ox pancreas, by means of alcohol, an extract which, when injected subcutaneously into animals, could remove the hyperglycemia and glycosuria produced by administering epinephrin. Zuelzer also administered this extract to eight diabetic patients and in five decidedly favorable results were obtained. The sugar nearly disappeared from the urine; acetone bodies became much less; the patients improved in general condition and it looked, in 1908, as if the antidiabetic hormone had at last been discovered. Unfortunately the extracts also produced toxic effects and in the hands of others, particularly of Forsbach, working in Minkowski's clinic, these toxic effects so overshadowed any beneficial action of the extract that further treatment by this means was abandoned. This must be looked upon as an abandoned research, although there can be no doubt that Zuelzer, in 1908, came very near to isolating what we now call insulin.

Another of the researches I wish to call your attention to is that of my fellow townsmen, Rennie and Fraser. Rennie, a zoologist in Aberdeen, in confirmation of the work of Diamare, demonstrated that in certain of the bony fishes there are few if any Isles of Langerhans in the pancreas but that cells like those of the isles are collected in separate glands situated in the mesentery at a considerable distance from the pancreas and can be easily removed as intact glands. They are known as the *principal islets.* Rennie with Fraser then attempted to study the effect of extracts of the principal islets in diabetes. The extracts were administered by mouth, or the patients took raw glands, but without any definite effect on the diabetic symptoms. In one case, an extract was injected subcutaneously, but the toxic reaction was so marked that further investigation was abandoned. These investigators, like Zuelzer, very nearly caught the elusive hormone but they just missed

doing so because they were frightened by toxic symptoms, the cause for which they did not understand. The repeated failures to prepare pancreatic extracts that could relieve the symptoms of diabetes and be safe for repeated administration to man seemed to discourage any further attempts in this direction and the search for the pancreatic hormone now came to be the problem exclusively of the physiologist and biochemist. To this group of experimental investigators, whose work I will now describe, we owe a very great deal for adding a sufficient amount of positive evidence so that hope was not abandoned, that at some time the pancreatic hormone might be discovered.

The first research of this group was that of Knowlton and Starling, who in 1912 published results which attracted a great deal of attention. They stated that the rate at which the isolated heart of the diabetic dog consumes sugar is much less than that of a normal animal. Their experiment consisted in rendering a dog diabetic by extirpation of the pancreas then perfusing the heart and lungs with Locke's solution containing dextrose. By estimating the amount of dextrose in the perfusion fluid from time to time they calculated the rate at which dextrose is used by the heart, and they concluded that this rate was much less for the diabetic heart than for the normal heart. This would show that the fault in diabetes depends on the fact that the tissues have lost the power to oxidize carbohydrate. They also stated that when a pancreatic extract, made with weak acid, was added to the fluid perfused through the diabetic heart, sugar came to be used up at the same rate as in the normal heart. Other observers in repeating Knowlton and Starling's experiment did not obtain the same results. Starling and various collaborators afterwards pointed out that a considerable disappearance of sugar occurs in the lungs in such a heart-lung preparation as

was used in these experiments and that the results were therefore inconclusive. The subsequent investigations of Starling and Evans, on the respiratory exchange in heart-lung preparation, are, however, of great importance in showing that oxidation of sugar is interfered with in the diabetic heart.

The next investigator I will mention is E. L. Scott who in 1912 prepared alcoholic extracts of the pancreas and showed that they could sometimes relieve certain of the symptoms of diabetes in animals. Toxic symptoms, however, developed and the investigations were not carried further. Another worker is Murlin who prepared alkaline extracts of the pancreas, and also of the duodenum, and demonstrated that they reduced the degree of glycosuria in diabetic dogs. Finding, however, that the latter effect could also be obtained by the administration of alkalies alone, the investigation was abandoned, except for some observations on the respiratory quotient, until after the first publication of the initial experiments of Banting and Best in Toronto. Murlin made the mistake, in my judgment, of using the wrong medium with which to extract the pancreas. Although insulin is stable to a certain extent in alkali, it is less so than in other reactions, and Murlin's failure to demonstrate the presence of the pancreatic hormone is partly due to the fact that he used alkali in the method of extraction. Moreover, he fell into the same error as others in that he usually confined his attention to the study of one symptom at a time and did not demand evidence obtained by the observation of several symptoms. I think there is no more common mistake in modern medical science than to base conclusions on results obtained by observing one symptom, or one change in a physiological function. Before any final conclusion is permissible regarding the action of an extract, I believe, one should always insist upon observing the effect

on two symptoms at least and these two symptoms should, so far as possible, pertain to distinct and separate functions.

Kleiner showed that weak saline extracts of fresh pancreas when administered slowly to diabetic animals usually caused a decided reduction in the percentage of blood sugar and this was shown not to be dependent upon dilution of the blood with water. Mention should also be made of the work of Paulesco published in France in 1921 in which it is claimed that extracts of the pancreas—the exact method of preparation of which he does not give in detail—lowered the percentage of blood sugar and urea in the blood and diminished the sugar excreted in the urine of diabetic animals.

The suggestive evidence of the existence of the pancreatic hormone afforded by such experiments as these led A. H. Clarke, working in the laboratory of W. G. MacCallum, in Baltimore, to attempt the demonstration of the pancreatic hormone by an entirely different principle. Clarke perfused the excised pancreas of an animal immediately after death with Locke's solution and then he perfused this fluid through the heart and found that the rate of consumption of sugar by the heart was much greater than it was when fresh Locke's solution was perfused. This result seemed to demonstrate that the pancreas yields up something to the fluid circulating through it which enables the heart to consume sugar more readily, and I have considered Clarke's experiments as absolute evidence that there is an internal secretion of the pancreas and that its isolation was only a matter of diligent and persistent investigation. It should also be pointed out that although Clarke did not find that the reducing power of the Locke's solution was changed by perfusion through the pancreas alone, he did find that a remarkable alteration occurred in the polarizing power of the sugar. In light of the recent

work of Hewitt, Pryde and de Souza and of Winter and Smith it would appear that the α and β glucose present in the Locke's solution before perfusion became changed into γ glucose which is much more readily utilized by the tissues. Either the internal secretion of the pancreas caused this change or it was produced in the pancreatic cells.

The More Recent Experimental Work upon the Pancreas

Now we have arrived at the stage of our brief survey of the subject at which the more recent work in the outline of these lectures may be said to start. The initial step in this work was the observation made by Banting and Best that simple extracts of the residue of the pancreas that remains some weeks after ligation of the duct of the gland in a dog is capable, when injected subcutaneously or intravenously into diabetic animals, of alleviating the two principal symptoms of diabetes, hyperglycemia and glycosuria. They also found that the general well-being of the animal was improved and that it lived longer. The animal after depancreatectomy usually lasts only a few weeks, but Banting and Best were able to show that under the influence of their pancreatic extracts it could be made to survive many weeks longer. Further details of the subsequent investigations on diabetic animals I propose to defer until tomorrow's lecture.

The Significance of Banting and Best's Experiment

To appreciate the significance of Banting and Best's experiment in connection with the problem as a whole, I propose in this lecture to describe briefly the structural relationship and characteristics of the islet tissue and then to review the experimental evidence which sug-

gested the possibility that the duct-ligated pancreas might prove a satisfactory source of material from which to prepare extracts containing the antidiabetic hormone. Embryologically, the islet tissue, like the acinar, is derived from outgrowths of the primitive alimentary tube (pancreatic anlage) and as development proceeds, the differential characteristics between the two kinds of cells come to be: first, the development of granules, characteristic for each kind of cell, and second, the separation of the islets from the acini.

In attempting a description of the structure of these tissues it is important at the outset to have clearly in our minds how we are to differentiate between the islet tissue and the acinar tissue. In the investigations of the earlier anatomists the isles were really distinguished by their negative characteristics, that is to say, by the fact that they did not contain the zymogen granules which, as you know, are a conspicuous feature of acinar cells. As R. R. Bensley, Professor of Anatomy in Chicago, has pointed out, the islets were distinguished by what they did not possess and not by any positive characteristics. In this way, as he points out, the investigators have exposed themselves to the risk of including in the category of islets many acinar cells from which, by oversecretion or other means, the stores of zymogen granules have become exhausted. Obviously, there is danger of error in differentiating anything by its negative characteristics. This loose definition of islet cells is now replaced by one of a positive nature elaborated to great perfection through the careful histological work of R. R. Bensley and his school, and which depends, partly, on the demonstration in the islet cells of intracellular granules staining differently from those of the acini, and partly on differential, *intravitam,* staining of the islets as a whole. For the differentiation of the granules the pancreas is removed immediately after

death and small portions placed either in a watery or an alcoholic solution of chromic acid sublimate. The sections are stained with neutral gentian violet, and it is found in those fixed in the watery solution that granules are deeply stained in most of the cells of the islets, and that the zymogen granules in the acini are also stained, but of a different tint. In alcohol preserved material, on the other hand, most of the zymogen granules have dissolved and in the islets only a few cells show stained granules which are also of a different tint from the granules stained in the tissue fixed in the presence of water. Lane, who elaborated the technique, has called the cells of the islets whose granules are soluble in water, the α-cells (i. e., they are stained in alcohol-preserved material) and those soluble in alcohol the β-cells (stained in water-preserved material). Other varieties of islet cells have been described, but there are only two of these which are apparently of importance. Recently Lane's and Bensley's technique has been considerably simplified, especially by Martin, so that both these varieties of cells can be differentially stained in tissue prepared in the same fixative.

The α-cells are only seen here and there in the islet, often at the periphery, on which account they have been held by some to be transition forms between the islet and acinar cells. There is no evidence of this as I will show later. There is no evidence for the claim that the islet cell is derived from the acinar cell; the two are distinct and separate. They have no relation whatever except that they come from the same embryological source.

You can easily see how the pathologists' interest would be aroused to ascertain the exact variety of islet cell that undergoes histological changes in diabetes. As you know, various investigators, including Allen, Homans, etc., in this country have pretty conclusively

shown that in diabetes the lesion is in the β-cells and it is altogether likely therefore that these cells are the source of insulin. This we might infer from the facts that they are soluble in alcohol and that no other agent is more useful for dissolving out insulin from the pancreas than weak alcohol.

Now, I will show you slides of preparations which will illustrate some of the facts I have been trying to describe. In Figs. 1 and 2 are shown sections prepared by Dr. Slater Jackson from the pancreas of the skate, which is representative of the so-called cartilaginous fishes (Elasmobranchi), and you will observe that the Isles of Langerhans are imbedded in the pancreas as is the case in the higher vertebrates. The islets, however, are particularly interesting in the skate because they show a very close relationship to the ducts, some of which are shown in cross section, others in longitudinal section. Only occasionally do you find islets that are not visibly associated with ducts.

In the more highly magnified portion of the same section, you can see very clearly how the islet, as it were, grows from the duct. In the duct there are always two layers of cells and from the outer layer of these the islet juts out, but it seldom juts out very far in the pancreas of this animal; it always remains more or less related to the duct. It is often encapsulated. The islet cells are mainly of the β variety. The secreting acini are seen to be very darkly stained, the cells being packed full of zymogen granules.

A more highly magnified portion of the skate pancreas demonstrates the close association between the islet and duct tissue still more plainly, and the islet which is shown is seen to be composed almost entirely of β-cells with three α-cells at the upper portion.

Besides the general difference between the granules

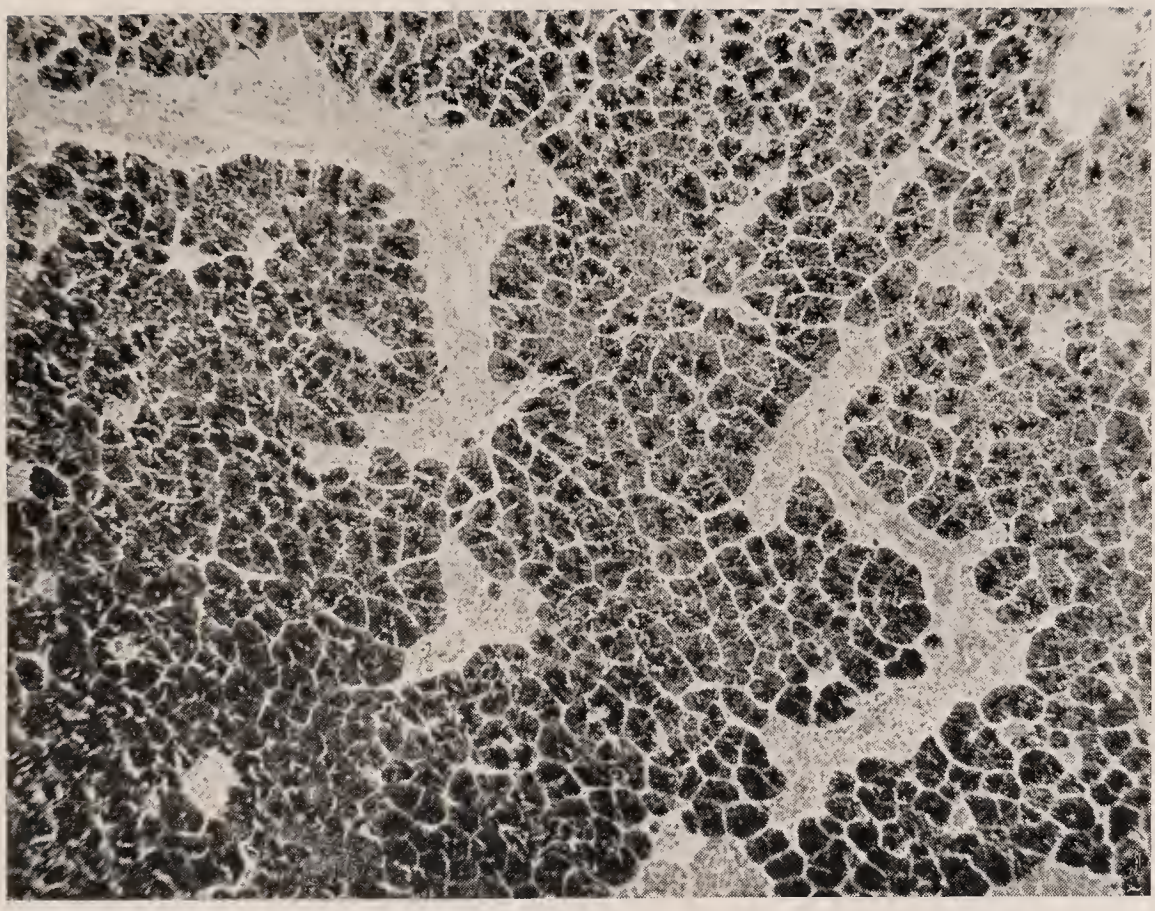

Fig. 1.—Section of pancreas of skate X 70. Iron hæmatoxylin and congo red. Note the close relationship of the islet tissue to the ducts. (From Slater Jackson, Jour. Metabolic Research, 1922, ii.)

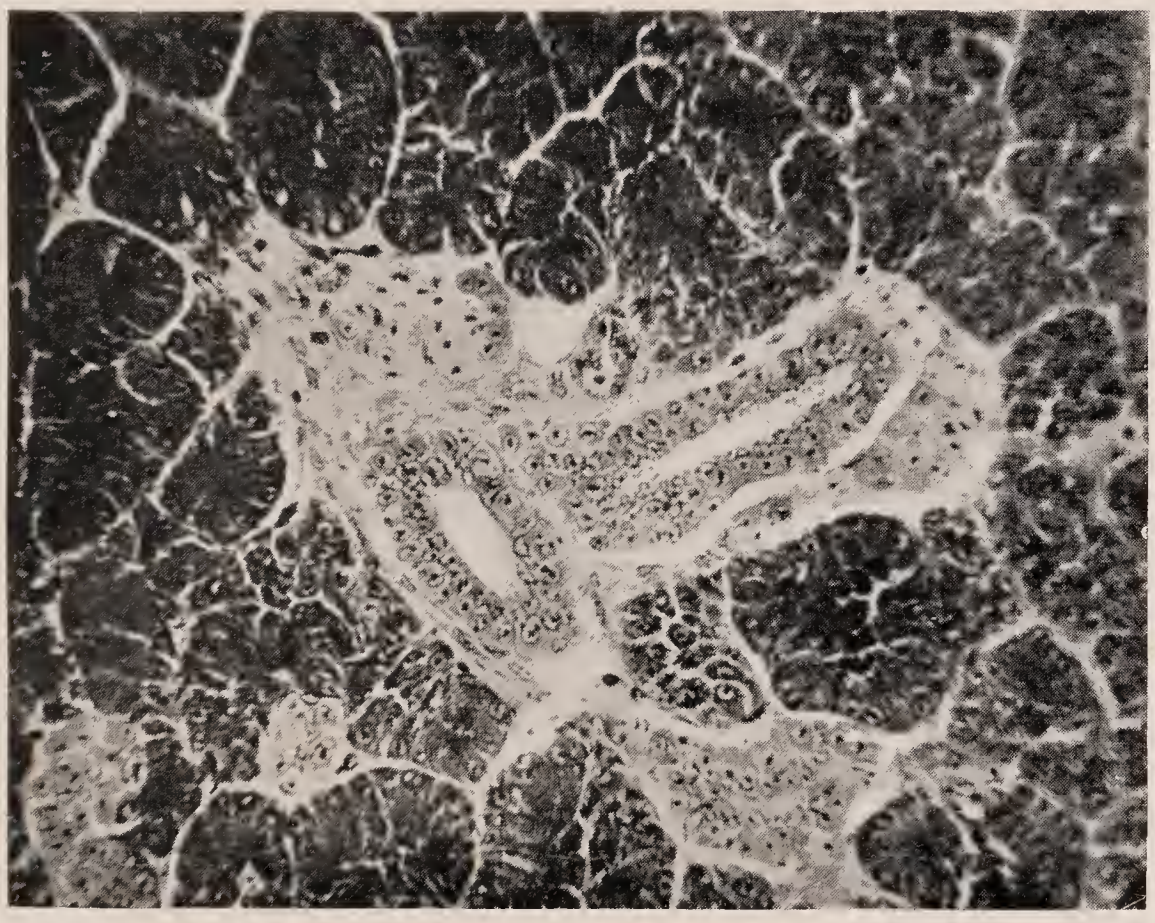

Fig. 2.—Same slide as that of Fig. 1, X 320. Note the continuity of islet tissue with the outer layer of cells of the duct. (From Slater Jackson, Jour. Metabolic Research, 1922, ii.)

of the secreting cells and those of the two varieties of islet cells, there are also other cytological characteristics which are clearly revealed in preparations stained with fuchsin. The point I want to emphasize is with regard to the mitochondria. These are present in all cells, but in those of the ducts and islets they exist as rod-shaped bodies which can be seen scattered throughout the protoplasm, whereas in the acinous cells, they are arranged as filaments, which give to the clear protoplasm of the cell a striated appearance. In the islet and duct cells, also, there is no chromidial substance which, along with zymogen granules, is a marked feature of the acinar cells. The latter also possess a large oxyphil nucleolus which is absent in the islet cells. Using these positive characteristics, Bensley and his collaborators have definitely shown that there is no structural relationship between the cells of the acini and islets. Occasionally an islet cell may occur, isolated among the cells of the acini, but apart from this, the two groups of cells occupy different positions. As I pointed out earlier in the lecture, the chief evidence upon which rests the claim that the islet tissue may originate from the acinar is that the latter is markedly increased under certain conditions, such as starvation, or the repeated injection of secretin. When the positive characteristics for each kind of cell are used to differentiate the cells, however, the islets are seen to be independent structures, although they are derived from the duct epithelium, as are also, of course, the acinar cells.

Bensley's Method of Counting the Islets

We may now pass on to an entirely different method for the identification of islet tissue, which is also to be credited to R. R. Bensley, and which has proved of great value in experimental work, namely, that of count-

ing the islets after staining them by intravitam methods. That does not mean that the dye has to be injected while the animal is still living, but it must be done before too much coagulation has taken place in the blood to prevent a reasonably free circulation. If such a dye as janus green or neutral red be injected into the aorta of a small animal, or in a suitable artery of the pancreas of a larger one, it will at first stain all the structures of the pancreas. Janus green, for example, will stain the whole gland at first a deep blue but by covering with a cover slip the dye will become reduced to a red color in the acini more quickly than in the Isles of Langerhans. These come to stand out as bluish particles and by taking portions of the gland and pressing them out beneath cover slips, one can count the number of islets and in this way definitely state whether or not these have become increased or decreased in number. Bensley has actually counted the islets in the entire pancreas of small animals. For instance, in a guinea pig, in which the pancreas weighs a few grams, there may be about 25,000 islets—a very large number—and one can imagine how many islets there must be in the pancreas of man. It represents a lot of tissue. Looking at the section of pancreas stained by ordinary methods one may form the impression that the islet tissue is so scattered that it cannot be of much importance. When examined by the intravitam method, however, it is seen to be relatively abundant. It is of interest that, by this method, Bensley has been unable to show that there is any constant difference in the number of islets in the different parts of the pancreas. Usually somewhat more were found in the splenic than in the duodenal end, but there were not the striking differences that histologists, working with older methods were inclined to think there were.

Relationship of Islets to the Duct System

Still one other detail of structure requires attention, and this is the relationship of the islets to the duct system of the pancreas. The details of this relationship we again owe to Bensley who demonstrated the ducts by vital staining with pyronin or with methylene blue and demonstrated "a system of epithelial tubules of great complexity which form a web of anastomosing ductules around the main duct and its branches." From the tubules, acini and islets are each separately developed and the islets can be seen in all stages of development. This is a fact of evident importance in connection with diabetes, for it suggests that it may be possible in adults to have regeneration of islet tissue after a great part of this has become destroyed by disease, as in diabetes. In this disease there is no doubt but that much of the islet tissue is destroyed and that destruction of the remainder is being accelerated by the overstrain it is put to in order to produce enough of the hormone, insulin. Is it possible, if this strain be taken off, either by diminishing the demands of the organism for the hormone by dieting or by supplying it as insulin from without, that new islet tissue will have a chance to become regenerated? The fact that islets may develop from the ductule epithelium suggests a favorable answer to these queries. The arrangement of the ductules shows branching into an anastomosing system, and the islets hang like grapes on this system of branches. The lumen of the ductule is always separated from the islet cells by duct cells, although into the larger islets the lumen may penetrate for a short distance. These facts explain why relatively few islets are to be seen toward the surface of the lobules, and at the same time they explain why the islets may sometimes be seen very closely related to the acini.

The Blood Supply of the Islets

Finally with regard to the blood supply of the islets: This has been demonstrated by injecting the vessels with carmine gelatine. If the methods of differential staining for islets be also employed, it can be shown that the larger islets have one or more large arterial branches running to them, the capillaries being unusually large and arranged in a glomerule-like fashion so that they come in close association with the cells. It is an interesting fact that in the principal islets of bony fishes the blood supply is also extraordinarily rich. *It is a significant fact that the glands of internal secretion like the thyroid, the suprarenal, and the Isles of Langerhans, have this extremely rich blood supply.*

The Comparative Anatomy of the Pancreas

I have taken you perhaps more into details than you bargained for in this field of anatomical structure, but I hope that here and there I may have shown some practical application of the things that have been found. Now I am going to venture to take you even a little further, namely, into a field of comparative anatomy. The work in this field originated with the observations of Diamare and Rennie, who as I have already told you, studied the anatomical relationship of the "principal islets" in various of the bony fishes. Rennie found these structures constantly present in certain groups, particularly in two fishes that are very abundant, namely, the angler fish and the sculpin. The angler fish (Lophius piscatorius) is one of the ugliest monsters of the sea, weighs about 35 pounds, and is shaped somewhat like a huge tadpole with an enormous head tapering into a long narrow tail. It lies at the bottom of the sea and is called the angler fish because there projects upward from the head a little mast provided at its free end with

a tuft of barbels which float in the water, and other fish, thinking this to be bait, come down; the huge mouth opens and the fish is gone. Lophius is often caught in deep trawls all over the world, but mostly in temperate zones and it is a great nuisance to fisherman except, it is said, by some who when they bring them aboard do not throw them back until they have opened them to get the fish out of the stomach, to be sold as fresh fish. In Lophius the pancreas does not exist as a separate gland, but is spread out as narrow bands of pancreatic tissue along the branches of the portal vein. The Isles of Langerhans on the other hand are mainly, if not entirely, collected in definite and encapsulated nodules, "principal islets," located in the mesentery always in the same position and with a very rich blood supply.

The position of the largest of these islets is close to the anterior pole of the spleen near to the conspicuously long cystic duct. There is another principal islet nearer the pylorus and many other smaller ones scattered in the mesentery near to it. In all varieties of fish examined the largest of the principal islets was always found to lie near the spleen. You can find it readily if you find the spleen. In certain fish such as the trout, salmon, haddock, etc., the pyloric caeca are very numerous and the islets that exist are very small, usually microscopic structures embedded in the fat which lies between the caeca. Sometimes these islets are quite free of acinar cells but frequently both exist together, although quite distinct and separate.

Little can be said as yet regarding the cytological characteristics of the cells composing the principal islets. The sections from which the available drawings were prepared were all copied from Rennie's work which was done some fifteen years ago, before modern methods of staining were used and you cannot see anything of the cells—they are broken down. Dr. Slater Jackson of

McGill, who worked with me at St. Andrews during last summer, has collected a lot of this material for histological study, but I have none of these slides to show at present.

The Structural Changes in the Pancreas after Ligation of Its Ducts

This brings me to the end of the anatomical details and leads to a discussion of the experiments from which the work that I am going to tell you of tomorrow originates. The anatomical considerations which I have just discussed indicate a more or less independent function for the acini and islet tissue, but they do not definitely show us what that function may be. This suggested to many investigators that it would be interesting and important to study the structural changes in the pancreas following ligation of its ducts. One would expect, after ligating the ducts, that the acinar cells, which presumably produce the external secretion, would atrophy much more quickly than the islet cells which are not so closely related to the ducts. Such has proved to be the case, in a general way. After ligation of the ducts the acini degenerate much more rapidly than the islets, but it takes a long time before all the acinar tissue has disappeared because of the extremely interesting train of events that follow, namely an initial degeneration, then regeneration, followed again by degeneration. For example, after ligating the ducts in a rabbit, in which the experiment is comparatively easy, because there is only one large duct, the following results, of work by Clark, are described by Bensley in his Harvey lecture. In the guinea pig seven days after ligation of the ducts most, but not all, of the acinar cells have degenerated and in those which remain, and also in the duct cells, there is the start of regenerative changes. Toward the end of

the first month (in the rabbit) the regenerative change is prominent, "resulting in the formation of new acini and new islets of Langerhans from the remains of the duct system." The smaller islets are involved in the initial degenerative process and by the end of the first month so also are the larger ones, and connective tissue invades the islets so that the cells show some atrophic changes. "Thus, both primary tissues are present in the pancreas for a certain period and throughout this period acini are being constantly formed, pass through a series of developmental states and to some extent undergo atrophy or become again de-differentiated. At the same time the islet tissue is being progressively increased by the addition of new islets developed from the duct system with which they remain in continuity and the original islets for the most part atrophy as a result of the invasion of connective tissue. In five and one-half months all of the original islets have disappeared and the new tissue exists as branching masses, giving all the microchemical tests for islet tissue." Even at this late date there are also acini, the cells of which contain zymogen granules. After this date the acinous tissue gets less and in 533 days after the ligation, in one rabbit, the residue of pancreas consisted entirely of regenerated islet tissue. The significant facts brought out by this work are that it takes such a long time for acinous tissue finally to disappear and that islet tissue also degenerates and then regenerates.

These researches of anatomists on the effect of ligation of the duct immediately suggested to experimentalists the interest of finding whether an animal in which the duct had been tied would develop the symptoms of diabetes, and as you know, most interesting work in this connection has been done by W. G. MacCallum. One of MacCallum's best experiments was to isolate a portion of the pancreas from the main gland by ligating

it, and then, in seven months, to remove the main gland, leaving the isolated portion. The animal still retained the power to metabolize considerable quantities of carbohydrate after this second operation, but it lost this power after a third operation in which the remnant was removed. The remnant was found on histological investigation to contain only islet and duct tissue. This work shows that complete isolation of portions of the pancreas in the dog does undoubtedly cause complete disappearance of acinar cells with retention of those of the islets some months after, and that the animals retain the power to metabolize carbohydrate, from which the conclusion may be drawn that it is the islet cells which furnish the internal secretion.

It is on the basis of these observations that Banting and Best's original experiments depended. Assuming that the failure of previous investigators to secure pancreatic extracts that would with certainty contain the antidiabetic hormone depended upon the destruction of this by the proteolytic ferments necessarily also present in the extracts, Banting suggested making extracts from the duct-ligated gland, and with Best's collaboration succeeded in showing that these markedly lowered the blood sugar and reduced the urinary sugar in diabetic (depancreatised) dogs. Dr. Banting is to give you the details of this work tomorrow evening, and all I wish to say at this time is that the definite nature of the results afforded conclusive evidence to me that the elusive antidiabetic hormone had at last been discovered and a vast new field of exploration in metabolism thereby opened up. Those of us who have been fortunate enough to be associated with these two explorers when they blazed the trail into this new territory have done our best to build a solid pathway and some of the things that have been found by following it, regarding the influence of the newly-discovered hormone on carbohy-

drate and on fat metabolism, will form the subject matter of tomorrow's lecture.

It may be the case that all of the acinar tissue was not degenerated in the tissue from which Banting and Best made their extracts. But what of it? For evidently enough was destroyed to reduce the autolytic enzymes to such a degree that the hormone was sufficiently preserved to permit of its presence being identified with certainty.

The Source of Insulin

Finally let me say a few words concerning the source of insulin. Although the experiments and anatomical facts which I have related in this lecture furnish strong circumstantial evidence that the islet cells are the source of the pancreatic hormone, there has until recently been no evidence of a direct nature. The absence of glycosuria in animals from which all acinar cells have been caused to disappear by duct ligation does not necessarily show that the remaining islet cells produce an internal secretion, since, as already explained at the beginning of the lecture, the effect might be the result of a local action of the blood while this is circulating through the remnant of the gland. Neither do the experiments of Banting and Best afford this evidence, since there were probably acinar cells in the tissue from which their extracts were made. Satisfactory evidence can alone be secured by making extracts from islet tissue that is entirely free of acinar cells and comparing its effect with that of other extracts prepared from tissue containing no islets but only acini. These conditions are probably fulfilled in the bony fishes already referred to. Although the histological work of Professor Jackson is not as yet sufficiently far advanced to determine the extent to which the principal islets are devoid of acinar cells, or the pancreas itself of islets, there is rea-

son to believe that the former are overwhelmingly composed of islet cells and the latter of acinar, thus making it of interest to search for the presence of insulin in extracts separately prepared from the two sources. This was done during last summer at the Biological Station of the Biological Board of Canada at St. Andrew's, N. B. and it was found that insulin is abundantly present in extracts of the principal islets but entirely absent from those of the pancreas itself. Indeed the extracts from the latter source gave evidence of containing some substance which acts like epinephrine in causing an excess of sugar to appear in the blood. To give the evidence for this statement, I must anticipate the first part of tomorrow's lecture by stating here that the laboratory test for insulin is to observe the behavior of the blood sugar in a rabbit following the subcutaneous injection of an extract supposed to contain it. The following results show the effect produced on the blood sugar by injecting the extract that was made from the principal islets of the sculpin. The normal of blood sugar to begin with was 0.102 per cent. At 11:30 injection was made. At 12:10 the blood sugar was 0.070 per cent, at 1:30 it was 0.058 and at 3:50, 0.063 per cent. In the second experiment the extract was made, not from the islet, but from the pancreatic tissue. At 10:45 A. M. the blood sugar was 0.110, at 2:45 P. M., 0.156. These two experiments are representative of many others in which it was definitely shown that the insulin comes from the principal islets which are homologous with the Isles of Langerhans of higher animals and not from pancreatic secreting cells; the latter, on the contrary, appear to contain a substance which raises the percentage of the blood sugar.

BEAUMONT LECTURE II.

BY PROFESSOR J. J. R. MACLEOD

January 30, 1923

Experimental Results from Insulin

I propose to review the results of the experimental investigations which have been made with insulin and which, in our judgment, justified our recommending the use of this material in the treatment of diabetes in man. Some of the experimental results which I am to detail were obtained side by side with others obtained in the clinic, but let me assure you that the clinical application of this work could never have been undertaken had the experimental investigations not preceded it. The lowering of the sugar concentration in the blood and urine would not alone have justified clinical trials with insulin, since several observers, such as Zuelzer, had already shown that pancreatic extracts of a similar nature to insulin could do this. It was necessary to carry the investigations far enough in the laboratory to demonstrate the exact nature of the other physiological changes brought about by insulin and to show how toxic effects could be avoided or counteracted.

In discussing the effects produced by extracts of the principal islets of the pancreas in certain bony fishes, I explained that the most useful test for the presence of insulin consists in observing the effect which follows when this substance is injected subcutaneously or intravenously in normal rabbits. Collip found that very shortly after the injection the percentage of blood sugar

falls often to a very low level and then in an hour or so that it gradually recovers. We observed that when the sugar fell in a rabbit to about 45 milligrams per 100 c.c. of blood, certain very alarming symptoms developed. These, in typical cases, consist of convulsions in which the animals throw themselves about violently, turning over sideways with the head usually retracted, each convulsive seizure lasting for perhaps one to three minutes. Then the animal lies in a collapsed condition —unconscious, breathing rapidly (often periodically)— until after a few minutes, a second convulsive seizure comes on. The convulsive seizures alternating with periods of coma may last for several hours, the convulsions gradually becoming weaker and weaker until at last the animal dies of respiratory failure. These are among the most alarming symptoms I have ever seen in pharmacological or physiological investigations—definite, clear-cut, and usually associated with a percentage of sugar of about 0.045. Sometimes, it is true, the symptoms may appear before the blood sugar has reached this level, or more frequently they may remain absent until it has become much lower. On account of this frequent incidence of symptoms at about 0.045 per cent blood sugar, we have tentatively defined as a unit of insulin the amount which is capable of lowering the percentage of blood sugar to this level in a rabbit weighing 2 kg. within three hours. There has been a little confusion as to the exact definition of this unit on account of the fact that certain people have chosen lighter rabbits than those with which the test was originally made. When a very light animal is used, the number of units per cubic centimeter comes out distinctly higher, and this has caused not a little confusion, for the firm which was the first to manufacture insulin in this country unfortunately chose those lighter rabbits for their assays so that the unit of the commercial product is at present of lower

value than the unit originally established. Partly to prevent confusion, and partly on account of the fact that it does appear that in certain cases of diabetes the smaller unit is sufficient for the treatment of certain cases of diabetes occurring in children, we have agreed to accept this smaller unitage. It is one-third the value of the unit as defined above.

I have described the typical acute symptoms; there are certain premonitory symptoms as the blood sugar descends toward the 45 milligram level. One can see that the animal becomes highly excitable and it evidently becomes very hungry and will sometimes attempt to eat the woodwork of its cage, or anything that appears to it to be capable of relieving the hunger pains from which it seems to suffer. It is very interesting that these premonitory symptoms, objective though they be, are perfectly definite and are analogous with those which have been observed in man. When an overdose of insulin has been given to man, the first symptom as a rule is a feeling on his part of alarm, of fear that something is to happen, of nervousness and then, a little later, he is very likely to experience symptoms of cardiovascular disturbance—flushing, perspiration, a sensation of hunger—and then, if the condition be not treated, a stage is reached in which tremors are felt in the muscles. You may not see the tremors—they may be merely subjective sensations—and later the patient may become highly excitable and may lose his mind temporarily, become unconscious, with symptoms that are similar to those observed in animals, probably not to the extent of producing convulsions, but very like those of coma. The earliest of these symptoms may occur when the blood sugar is only reduced to about 0.070 per cent. The association of these symptoms with a level of 45 milligrams per cent of blood-sugar would suggest that the symptoms may be due to the disappearance of the sugar

from the body fluids. It is remarkable that the symptoms appear at this level since Mann and Magath of the Mayo Clinic have also observed that when the blood-sugar decreases to about the same level in dogs from which the liver has been removed, the animals develop symptoms that are closely similar to those observed in the rabbits injected with insulin. This correspondence would not, however, justify the conclusion that the symptoms are directly related to the reduction of blood sugar; one must determine the effect produced on the symptoms when sugar is injected into the animal, before any such conclusion could be drawn. When glucose solution is injected subcutaneously (2 gm. per kg.) the animal recovers within a minute or two; even though the symptoms may be extremely severe, the animal is practically back to a normal state in a remarkably brief period of time.

At a demonstration which I made recently before the Academy of Medicine of Toronto I was particularly anxious to be certain that the animals would show really acute symptoms and Mr. E. C. Noble, who has been more particularly associated with this work, injected a very large dose of insulin into two animals at a time preceding the meeting which we thought would be suitable to have them in good condition for demonstration; but unfortunately the animals went into convulsions much earlier than we had intended and by the time the Academy met one died immediately after it was brought into the room, and the other was in deep coma and evidently in a moribund condition. I thought it hopeless to attempt an injection of glucose into this animal but nevertheless did so, and it was very interesting that although recovery was slow, it was sufficient to convince everyone by the end of the meeting that the symptoms had been satisfactorily antidoted. The animal sat up, although still very drowsy, and next morning it was

apparently normal. During its recovery the coma gave way to alternating periods of convulsions, which gradually disappeared. Glucose is the only sugar which has this striking effect. Some sugars closely related to glucose, namely levulose and galactose, have a slight transient effect in relieving the symptoms but apparently they cannot save the animal. Of the ordinary sugars, neither cane sugar nor lactose has any effect whatsoever. Nor have such possible precursors of glucose as lactic acid or glycerol any antidotal action. The only sugar that can definitely relieve the symptoms is glucose. There can be no doubt, then, that the symptoms are dependent upon the lowering of the percentage of glucose in the blood. This does not mean to say that these violent symptoms are directly due to the disappearance of glucose as such from the blood; that would be rather an unscientific conclusion to draw. One must imagine that the immediate cause of the symptoms is the development of some toxic condition in the nerve cells, the prevention of which in a normal animal is dependent upon a certain percentage of glucose in the tissue juices. What this toxic agent may be, we cannot say. One thinks immediately that it may be something which causes a disturbance in the acid-base equilibrium in the nerve cells. With this possibility in mind we have studied the effect of acids and alkalies in affected animals but no evidence of a beneficial effect has been obtained. It is very interesting, as was pointed out by Professor Barany, when he saw a demonstration of these experiments in Toronto, that similar symptoms are temporarily produced in a normal rabbit by spinning it. By taking a normal rabbit, for example, and holding it against the body and then spinning around with it five or six times, the animal when laid on the floor develops exactly the same symptoms as occur when the blood sugar percentage has been lowered to 0.045. The symp-

toms in this experiment do not last long, but they are so very nearly the same as those produced by insulin as strongly to suggest that the lesion responsible for the latter must be one that acts on the vestibular apparatus. For the present therefore we are working on the hypothesis that the toxic substance, whatever it may be, develops in the nerve cells, including those connected with the vestibular apparatus. In the accompanying table the figures in the first column give the percentage of sugar in the blood of normal rabbits before insulin was given and in the second column the percentage after

TABLE

BLOOD SUGAR		AMOUNT	TIME AFTER INSULIN	SYMPTOMS	ASSAY (RABBIT DOSES)
BEFORE INSULIN	AFTER INSULIN	c.c.	hours		
0.148	0.038	4	4	Violent convulsions	1 plus
0.12	0.040	4	4	Violent convulsions	1 plus
0.13	0.062	2	2	None	½ plus
0.122	0.040	3½	2½	Convulsions	1 plus
0.125	0.040	3	2½	Convulsions	1 plus
0.146	0.054	2½	2	None	1
0.115	0.065	2½	2	None	½
0.155	0.062	3	2	None	½
0.120	0.066	2	2	None	½
0.130	0.055	2	2¼	None	½
0.130	0.060	2	2¼	Convulsions later	½
0.180	0.040	2	1¾	Convulsions	1 plus
0.140	0.060	2	2	None	½
0.120	0.060	2	2	Convulsions later	½
0.120	0.065	2	2	Convulsions later	½
0.130	0.065	1	1½	None	½
0.105	0.04	2	2	Convulsions	1 plus
0.135	0.056	2	1½	None	½
0.135	0.045	2½	2¼	Convulsions	1
0.120	0.045	1½	2¾	Convulsions	1
0.140	0.045	3	2½	Convulsions	1
0.11	0.045	1	2	Convulsions	1
0.11	0.062	3	1¼	None	½
0.165	0.045	10	2	Convulsions	1
0.116	0.062	2½	4	None	½
0.138	0.056	5	1	None	½
0.041	0.042	1½	4	Violent convulsions	1 plus

insulin. The amount of extract injected is indicated in the third column, the time the blood samples were taken in the fourth and in the fifth column whether or not symptoms developed. If you scan the table you will find that the symptoms occur pretty definitely when the blood is about 45 milligrams per cent. If you average the blood sugar values at which convulsions occurred, you will find that it works out very close to the above value. Sometimes, as in several of the cases shown in the table, convulsions were observed when the nearest blood sugar was about 60 milligrams, but in these cases there was a considerable interval between taking the blood sugar and the development of convulsions. It is very difficult, as you can well understand, always to manage to get the sample of blood just exactly at the minute the symptoms appear.

Insulin and Hyperglycemia

I may pass on to consider work for which E. C. Noble is mainly responsible, the effect of insulin on nondiabetic hyperglycemia. You are aware that hyperglycemia, that is, an increase in the percentage of sugar in the blood, may readily be brought about in the laboratory by various experimental conditions. One of the most interesting of these is puncture of the floor of the fourth ventricle of the medulla (piqûre). Claude Bernard discovered about 1846 that this operation was followed within half an hour in a normal rabbit by a very marked glycosuria which was later shown to be associated with a marked hyperglycemia. It is one of the most certain methods of producing experimental diabetes in animals. The only time it fails is when a starved animal is taken, and it fails here because of the low content of glycogen in the liver. The hyperglycemia and glycosuria last in a well-fed animal until all the store of glycogen in the liver has been exhausted. The effect of insulin on this

form of experimental hyperglycemia is very marked. In the curves given in Fig. 3, one set shows the behavior of the blood sugar following piqûre in normal rabbits, and here it is observed that the hyperglycemia is very striking indeed—the blood sugar in some of these experiments rising to 450 milligrams per cent. The other set of curves shows the results when piqûre was performed on rabbits injected with insulin; here the effect on blood sugar is seen to be greatly suppressed, sometimes entirely inhibited. In an animal under the influence of insulin, piqûre never raises the blood sugar sufficiently to cause glycosuria.

Another form of experimental hyperglycemia of great interest is that produced by the subcutaneous injection of epinephrin. In a well-fed rabbit the hyperglycemia is just as striking as that produced by piqûre. When the epinephrin is given to rabbits after insulin, however, the effect on blood sugar may be very slight indeed, the exact result depending, obviously, upon the relative amounts of epinephrin and insulin that you use (Fig. 4). Since we can accurately tell the dosage of epinephrin, Dr. Eadie is trying to assay insulin by finding how much would have to be given to prevent any rise in blood sugar following injection of a definite amount of epinephrin. This method may possibly prove to be a very accurate one for the assay of insulin. There is a physiological interest in these results since they demonstrate an antagonistic action on blood sugar of two important hormones. It is on this account that some investigators have suggested that the hyperglycemia following pancreatectomy may really depend on an unapposed action of epinephrin but there is no direct evidence in support of such a view.

Asphyxia also causes hyperglycemia. The asphyxia may be produced in a variety of ways, either mechanically by blocking the air passages, or by the action of

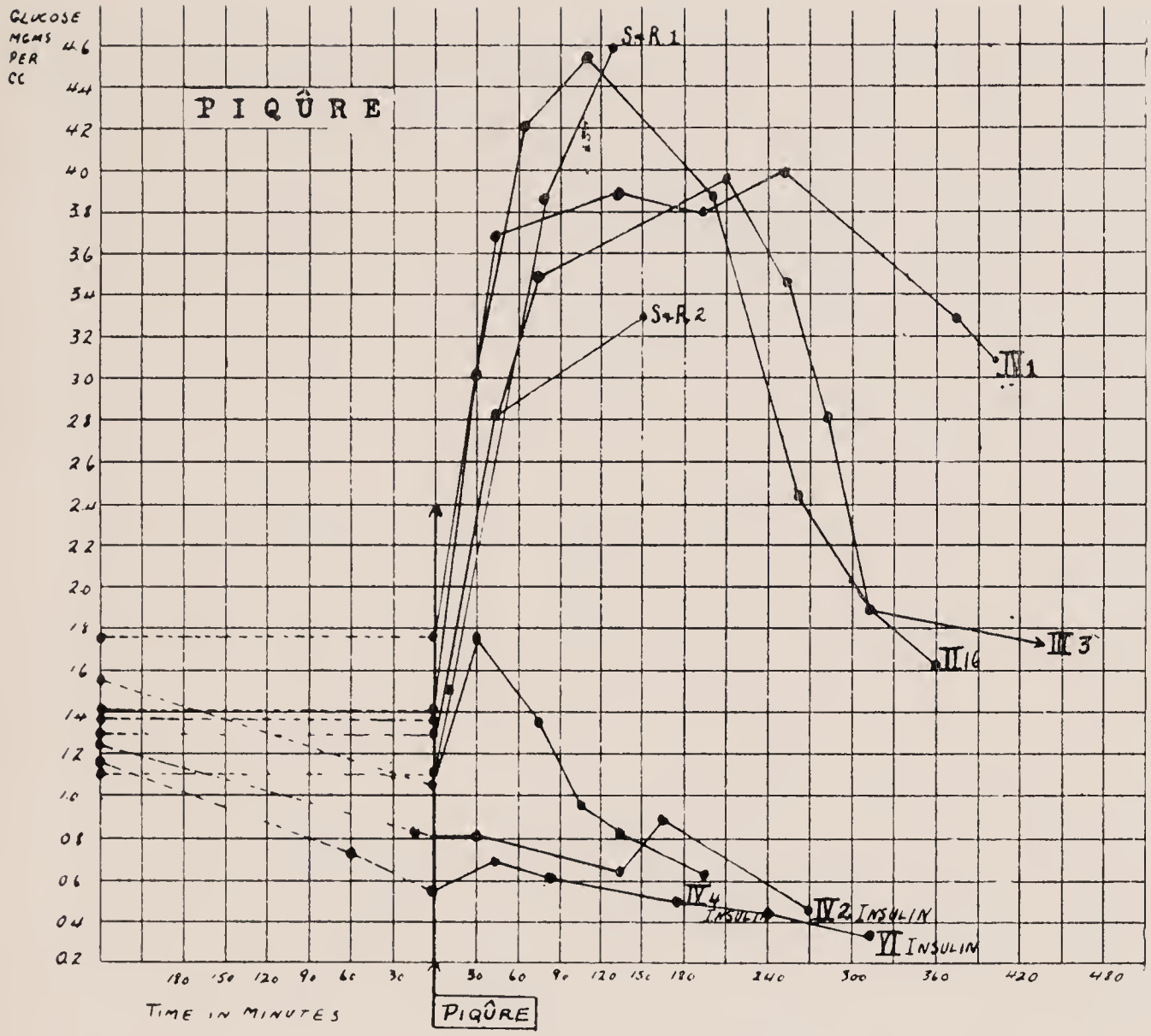

Fig. 3.—Curves of the blood sugar in rabbits after piqure. It will be seen that in the three cases in which piqure was performed after insulin, very little increase in blood sugar occurred.

Ordinates=glucose mg. per c.c.

Abscissae=time in minutes.

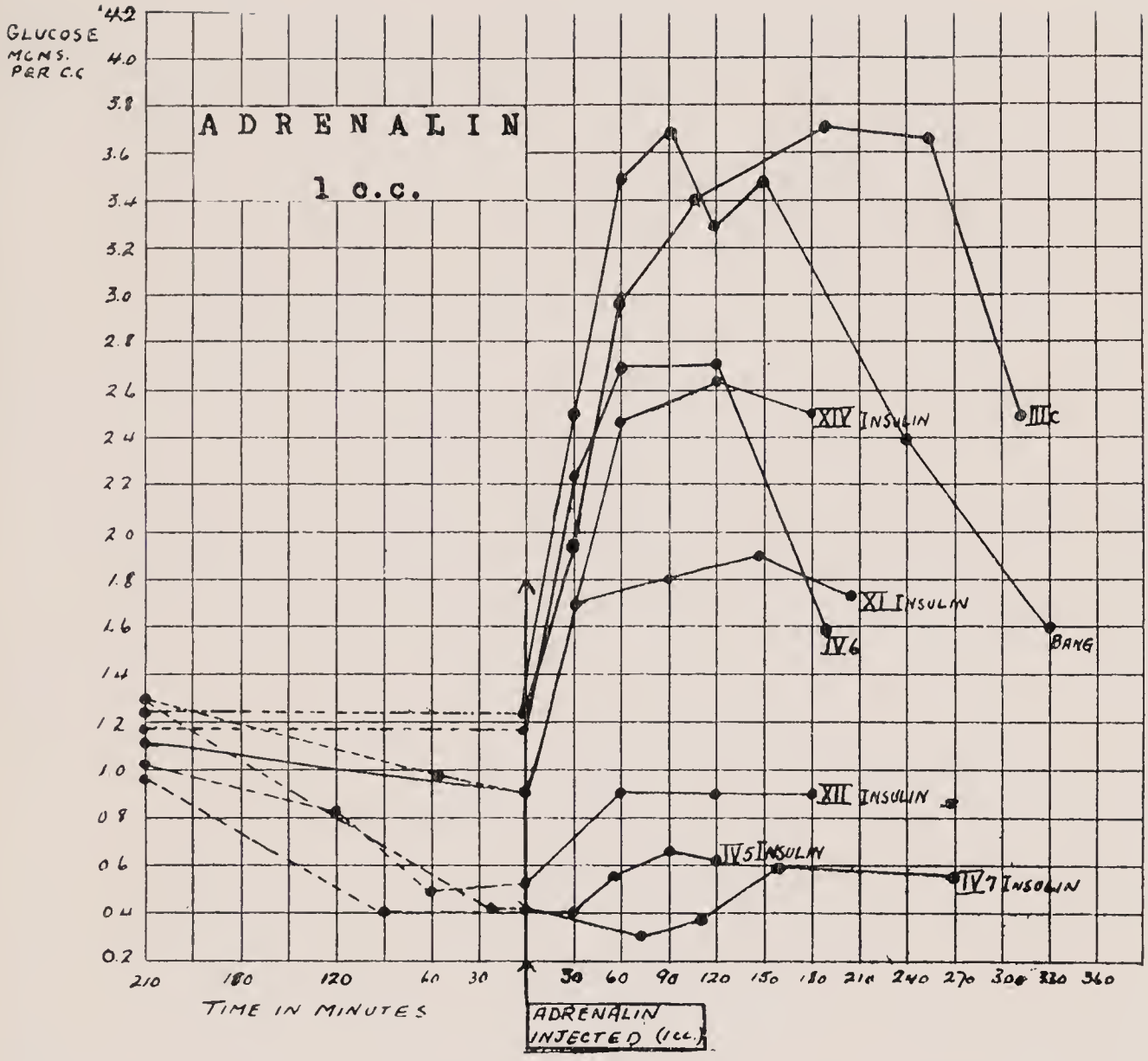

Fig. 4.—Curves of the blood sugar in rabbits following the injection of 1 c.c. of adrenalin. In certain cases insulin was also given and it will be observed that where this was of sufficient potency, as in curves XII, IV^{5}, and IV^{7}, hyperglycemia did not occur.

drugs, such as carbon monoxide (coal gas) or by the administration of anesthetics such as ether. It can be observed that when mechanical asphyxia is performed on "insulined animals," the effect on the blood sugar is very slight, never enough to bring about a condition of hyperglycemia.

For the experiments on carbon monoxide, the animals were placed in an air-tight chamber into which coal gas was delivered until the percentage of carbon monoxide was raised to a level known to produce marked hyperglycemia. The observations were then repeated on insulined rabbits, when only a slight elevation in blood sugar occurred.

With regard to ether, the results are equally pronounced. This result, obviously, has an application in clinical practice because it shows us that insulin will be useful to combat one of the dangers of ether, namely, its tendency to raise the percentage of sugar in the blood. This makes it likely that the surgical risk of operations on diabetic patients will be greatly lessened. Besides this clinical application, the result is of importance from an experimental standpoint, because it provides us with a method by which the blood sugar is prevented from rising as an effect of the administration of anesthetics. One of the great drawbacks to experimental work on diabetes in the laboratory has been the fact that *every known anesthetic inevitably produces hyperglycemia,* and this obviously masks the effects of other conditions on the blood sugar level. Insulin also greatly diminishes the rise in blood sugar which results from the ingestion or subcutaneous injection of glucose.

The Effect of Insulin on Diabetic Dogs

We see, then, that insulin reduces the percentage of blood sugar in diabetic (depancreatised) dogs, in normal rabbits, and also in rabbits that have been rendered hy-

perglycemic by various experimental means. It will now be convenient to describe the further effects of insulin on diabetic dogs. As I explained last night, diabetes of this type is produced by the total extirpation of the pancreas. If the gland be not all removed, the animal may become only partially diabetic, and, as you are aware, this partial form of diabetes has been a useful form to investigate experimentally, since it brings the animal into a condition not unlike that frequently seen in diabetes in man; that is, not complete diabetes, but a form that is amenable to dietetic treatment without which, however, it is almost certain gradually to develop into the fatal form. Within three or four weeks after complete pancreatectomy the animal dies of acute starvation, no matter how much food it may have taken in the meanwhile. During this period of three or four weeks the animal exhibits in an intensive form all the symptoms of the disease—marked hyperglycemia and glycosuria, acetone bodies in the urine, great hunger and thirst, serious skin lesions, abscess formation, etc. The only thing that is different in this form of diabetes observed in the laboratory and the diabetes observed in the clinic is that the acetone bodies are not nearly so abundant in the urine, and death is almost always due to marasmus, or acute starvation, instead of coma. Obviously such diabetic animals are very suitable for the investigation of the effects of insulin and were used, as I explained last night, by Dr. Banting and Mr. Best in their initial experiments on the effects of extracts of degenerated and fetal pancreas on the blood and urinary sugar. I also mentioned that these investigators observed that one animal lasted for a very much longer time than has been observed in the case of animals not treated by this method.

Let me now detail the effects of insulin on the other symptoms of depancreatised dogs. These I shall group

in three categories: first, the effect of insulin on the respiratory quotient, which is the ratio expressing the relationship between the amounts of carbon dioxide expired and of oxygen retained by the body in a given time. It is a ratio, not a quantity. It tells the kind of combustion going on in the body—not the amount. Let us imagine, for example, what would happen were the combustion in the body entirely of carbon. Then it is evident that the amount of oxygen which was used to oxidize the carbon would be exactly equal in volume to the amount of carbon dioxide which was produced, $C+O_2=CO_2$. This follows from the gas law which states that equimolecular quantities of all gases occupy the same volume. Let us consider next what the ratio would be when carbohydrates are being oxidized in the body. Carbohydrate is nothing more than carbon *plus* the elements of water, H_2O. Therefore, the oxidation of carbohydrate, chemically considered, amounts to the same thing as the burning of so much carbon, so that the quotient would be 1.0. In the case of proteins and fats it is different. In these foodstuffs there is not a sufficient amount of oxygen to oxidize all the hydrogen in the molecule and the formula in general might be said to be carbon *plus* hydrogen *plus* water. These substances require oxygen to combine with both the carbon and hydrogen, but the oxygen that combines with the hydrogen forms water which is excreted by other pathways than the lungs, so that the carbon dioxide which is excreted by the lungs must obviously be less in volume than the inspired oxygen. The respiratory quotient of fat and protein is therefore about 0.7 to 0.8, instead of 1.0. You can see quite readily from these considerations that a respiratory quotient of one in an animal would indicate that combustion of carbohydrate was taking place exclusively; that the only substance burning was carbohydrate. It is a re-

markable achievement, due very largely to the work of Rubner, in Germany, of Benedict, in Boston, and of Graham Lusk, in New York, that it should now be possible, by determining the respiratory quotient, to tell just exactly what proportion of carbohydrate, fat and protein a person is burning at a given time in his body.

Now let us consider the behavior of the respiratory quotient in diabetes. In general terms this disease may be said to be a condition in which the body has lost the power to oxidize carbohydrate. In the severe forms of the disease the respiratory quotient is always about 0.7 and it does not increase however much carbohydrate may be given to the patient or the animal. In milder cases a moderate increase may occur, so that the quotient furnishes a useful test for the intensity of the diabetic condition. Now, *it is extremely interesting that when insulin is given along with sugar to a diabetic animal, the quotient comes to behave immediately as it does in a normal animal;* it immediately rises toward unity. Let me quote some experimental results in support of this statement. Before the pancreas was removed in a dog, thirty grams of cane sugar by mouth caused the quotient to rise nearly to 1 in thirty-five minutes. The animal was then depancreatised and in seventy-four hours after the pancreatectomy it was again given twenty grams of cane sugar with the effect that the respiratory quotient only rose from 0.7 to 0.77. There was a distinct but very slight effect. A little later it was given the same amount of cane sugar, *plus* insulin, with the result that the respiratory quotient rose in fifty minutes to 0.91, and in one hour and thirty-seven minutes to 0.93. This is an absolutely convincing result and one that has been obtained repeatedly, not only in diabetic dogs, but also in diabetic patients. There can be no doubt, then, but that *insulin restores to the diabetic organism the lost*

power to oxidize carbohydrates. It makes it possible for the carbohydrate molecules to be utilized.

Let us now consider the effect of insulin on a process occurring at the other end, as it were, of the chain of carbohydrate metabolism. I refer to the deposition of glycogen. As you know, in a normal animal when carbohydrate is taken, large quantities of glycogen become deposited, particularly in the liver, and to a lesser degree in the muscles. In a diabetic animal, on the other hand, even though you feed it with large quantities of sugar, very little if any glycogen appears in the liver. The liver remains practically glycogen-free. *I have never seen more than 1 per cent of glycogen in the liver of a diabetic animal, however much sugar it may have been fed, whereas in a normal animal under these conditions 7 or 8, or even 20 per cent would be present.* On the other hand, in the diabetic animal the heart comes to contain a relatively higher precentage of glycogen than normal. Why this should occur we cannot tell. But it is an invariable fact and one, I think, of some significance. Now, *if you give the diabetic animal not only sugar but sugar along with insulin, glycogen becomes abundantly deposited in the liver* and apparently diminished in the heart. I have never seen so much glycogen in the liver of any animal as I have seen in that of a dog that was given large quantities of sugar along with insulin. In one case, Dr. Collip, who made the first analysis in this work, reported that he had obtained over 20 per cent of glycogen in the liver of one of these animals. It is almost unbelievable, but there is no doubt that the observation is correct. It is, as far as I know, the largest amount of glycogen ever reported to occur in the liver and quite often in other diabetic dogs treated with insulin we have observed from 8 to 12 per cent. Now, as you know, glycogen is the first step in the metabolism of carbohydrates, and the absence of

this material in the liver in a diabetic condition is obviously a thing of great significance—of a significance that becomes all the more striking when we note that the first effect of insulin is to cause glycogen to reappear. It would seem to indicate that glycogen is more than storage material, that *its formation is a fundamental step in the proper metabolism of carbohydrate.*

The Effect of Insulin on Fat Metabolism

Insulin also influences the metabolism of fat and there are two steps in this process that are involved. The first is the migration of fat. You are aware that *in diabetes the migration of fat becomes abnormal.* The liver, empty as we have seen of glycogen, becomes filled with fat, and the blood is very often loaded with this material so that it may become creamy in aspect; lipemia it is called. These symptoms—fatty liver and lipemia—are also observed in completely diabetic dogs, not so striking perhaps as in man, particularly the lipemia, but nevertheless quite evident, and easily detected. *Under insulin,* however, *the fat disappears from the liver just as glycogen takes its place and the lipemia very quickly clears up.* Therefore, the insulin has a very marked effect on the migration of fat. This matter requires further investigation. We have not had time to explore further this interesting part of our work, and we do not know exactly what it may lead to, but it is certainly significant that within a day or so of administration of insulin to a completely depancreatized dog, the whole plan of migration of fat should turn from an abnormal condition back to the normal.

The other step in connection with fat metabolism which insulin affects is its oxidation. Ordinarily, when fat is metabolized, the long chain of carbon atoms of which its molecule is composed, breaks down always at

the second carbon atom from the end of the chain, the β carbon atom, as it is called. From the long chain of the fatty acid molecule smaller groups containing 2 C atoms each are broken off until a molecule of only four carbon atoms is left (butyric acid) and then oxidation starts on this residue in the β position and under normal conditions it further breaks down into water and carbon dioxide. This last step is the most critical one of the whole process, the part likely to go wrong, for in diabetes oxidation sticks here, β-oxybutyric and aceto-acetic acids are formed and accumulate in the body along with acetone (acetone bodies) and cause toxic symptoms. These are probably due in part to the acid qualities of these substances, creating a condition of acidosis, and also possibly in part to the fact that they have a specific toxic influence.

These facts would indicate that for the proper oxidation of fat, particularly during its final stages, carbohydrate must also be undergoing metabolism. The two must oxidize together or, as it has been aptly put, "fat burns in the fires of carbohydrate." *If there be no carbohydrate fires, the fat smokes, and the smoke is represented by the acetone bodies,* which have a toxic influence. Now, in diabetes we cannot restore the oxidizing power of fat until we have stirred up the fires of carbohydrate combustion and this we may do by injecting insulin. Thus, in an experiment of January 6th, 1922, the excretion of acetone bodies by a diabetic dog was found by Collip to be 100 mg.—and on the 7th, 187 mg., the blood sugar at this stage being 0.035 per cent. Insulin was then given, with the result that the acetone bodies disappeared absolutely from the urine and remained absent for three days. On the fourth day they returned, 34 milligrams on this day, 55 the day after, and 114 the day after that. Insulin was again given and on the day following there were no acetone bodies.

The result is sufficiently conclusive since it shows not only that insulin caused a disappearance of the acetone bodies, but also that these returned some time after insulin treatment was discontinued.

Mechanism of Action of Insulin

Having described the effect of insulin on normal and hyperglycemic rabbits and on diabetic dogs, let me pass on now to what is perhaps the most difficult aspect of the work, namely, the cause of the action of insulin. How does it work? What is the mechanism of its action? The first step in such an investigation is to find out exactly what happens in the blood of a normal rabbit when insulin is given. This has been done by observing the blood sugar at frequent intervals in a large group of rabbits injected with varying quantities of insulin, the animals all being in exactly the same nutritive condition. That is to say, all the animals had been starved for twenty-four hours before the insulin was given, intravenously and in varying doses. It is seen that for the first half hour after giving insulin, the blood sugar comes down very rapidly so that it is at its lowest level in about half an hour after the injection has been made. That is extremely interesting, but still more so is the fact that within very wide limits of dosage the blood sugar comes down in different animals at exactly the same rate, the minimum being usually reached in thirty minutes. This parallelism in the rate of fall within the first half hour would seem to indicate that the action must be an intravascular one, occurring, that is to say, in the blood itself. This cannot, however, be the case because if you remove the blood during this stage you will find, on incubating, that sugar disappears from it at exactly the same rate as it does from normal blood under similar conditions; or, to put the

thing in technical language, the glycolysis curve of normal blood is exactly the same as that of blood removed from an animal recently injected with insulin. Similarly insulin does not alter the rate of glycolysis when added to blood outside the body. Another possibility is that the glucose might be condensed into less strongly reducing sugars, or into glycogen. This, however, cannot be the case because if you remove some blood when the sugar is at its lowest, and hydrolize it, (that is to say, heat it with weak acid solution,) the reducing power is not increased any more than is that of normal blood similarly treated. The action of insulin cannot, therefore, be intravascular, and we must conclude that the insulin passes from the blood through the capillaries into the tissue cells, in which it creates, as it were, a vacuum for sugar. Sugar, therefore, passes from the blood to fill the vacuum and it does so at such a rate that it is taken out of the blood more quickly than it can be replaced therein from the stores of glycogen in the liver. Hypoglycemia becomes developed because of the vacuum for sugar which has been created in the tissue cells.

What can be the cause for this development of a vacuum for sugar in the tissue cells? There are three possibilities: one, that it is due to an *increased combustion* of *sugar;* another, that it is due to condensation of sugar into glycogen; and the third, that it is due to a *reduction* of sugar into some other substance, possibly related to fatty acid. Let us see whether there is any evidence as to which of these is actually occurring. First, with regard to increased combustion; we have seen that the administration of insulin does restore the power of oxidizing carbohydrate in diabetic animals; is it not altogether likely, then, that the same thing will occur in the normal animal? It would, however, be dangerous to conclude that it does without further investigation,

because what occurs in a diabetic organism need not necessarily also occur in a normal organism, for in the latter case there may at all times be as much insulin available as is necessary for complete combustion of glucose. By observing the respiratory quotient, Dixon and Pember have obtained the very interesting result that in about half an hour to an hour after insulin is given to a normal dog or rabbit, the respiratory quotient definitely rises, and the oxygen consumption becomes greatly increased. The rise in the respiratory quotient would indicate that carbohydrate combustion is going on more energetically and the rise in the oxygen would indicate that there is a much greater energy exchange going on. One unexpected outcome of these experiments has been to show that frequently the respiratory quotient remains for a considerable time above unity during the action of insulin. This result must mean either that carbohydrate is being converted into fat, or that there is a blowing off of CO_2 due to the sudden development of an acidosis condition. In the latter, the respiratory quotient may temporarily rise above unity, because the acid breaks up the carbonates of the blood and produces a temporary excess of free carbon dioxide which is immediately got rid of by exciting hyperpnea so that it is blown off through the lungs. In several experiments the very high respiratory quotient has lasted for a much longer period than could be the case did it occur in this way, so that the rise in the quotient must indicate that besides the increased combustion of carbohydrates, a conversion of carbohydrate into fat is also going on. A similar increase of R. Q. to over unity, is also seen in hibernating animals in the fall months. During this time the animal is eating large quantities of nuts and other carbohydrate-rich foods, and it is storing away the carbohydrate in its tissues as fat so that the respiratory quotient rises con-

siderably over unity. This is the only condition in which such quotients have been observed in normal animals, and it is, therefore, extremely interesting from a physiological standpoint that a similar increase of the R. Q. occurs in normal animals that have been injected with insulin.

The third possibility, namely, that sugar is being deposited as glycogen, is supported by the observations to which we have already alluded, that insulin endows the diabetic animal with the power of depositing glycogen. If it does this in a diabetic animal, is it not probable that it will also do so in normal animals? That, it might appear, should be a very easy thing to prove, but let me assure you, this is by no means the case. Observations on the problem are being made by Miss O'Brien and Mr. McCormick, but the results do not warrant the conclusion that glycogen is being formed.

Summing up, the vacuum of sugar in the tissues would seem to be due to increased oxidation of glucose, relatively to that of protein and fat, and to its transformation by a reduction process into fat or fat-like substances. Both processes may possibly go on at the same time. If this be the case it suggests that the effect of insulin is, fundamentally, to convert glucose into some substance that is intermediate in metabolism between the three great groups of proximate principles. Evidence has quite recently been obtained by Winter and Smith in the biochemical laboratory in Cambridge, England, and by Hewitt and Pryde in London, which goes to show that glucose must first of all become converted into a highly reactive variety of glucose called γ glucose before it can be utilized by the tissue cells. This conversion of ordinary (α β) glucose into γ glucose may be the main function of insulin, possibly, as Winter

and Smith's experiments would seem to show, by its activating an enzyme which has this effect.

After the insulin effect has begun to wear off, perhaps because of its destruction or because of its excretion, the blood sugar begins to recover and the rate of recovery is in general largely in proportion to the amount of available glycogen in the liver. When there is little glycogen in the liver, as in starved animals, the recovery is relatively slow, whereas in animals that have been liberally fed with carbohydrate up to the time insulin was given, recovery is much more rapid. That recovery depends largely upon the glycogen stored in the body, has a practical application in the administration of insulin to man. It is obviously far safer to give insulin to patients who have already a good store of glycogen in the body, than to those that are glycogen-free. It is no doubt on this account that a dose of insulin that is quite safe for a well-fed rabbit will cause hypoglycemia in a depancreatised dog of many times the body-weight of the rabbit. *In practice, therefore, you will have to be most careful to see that symptoms do not develop in patients who are given insulin for the first time;* later on, when the glycogen stores have become fairly adequate, the dangers are likely to be less.

These important relationships of insulin to the glycogen stores of the animals have been shown in a slightly different way, namely, by observing the blood sugar of animals investigated in pairs, one animal in each case having a large store of glycogen in the body, and the other a very small one. When both animals of each pair were given exactly the same amounts of insulin, there was a striking difference in the results. In the case of the starved animal in the first pair, convulsions developed in less than two hours, while in the fed animal it took three hours for them to develop. The blood sugar came down to practically the same level in both cases, but

after attaining its lowest figure, it began to recover in the fed animal, whereas it disappeared almost entirely in the one that was starved. There was no tendency to recover, since there was no glycogen to draw on. In another experiment a somewhat smaller dose of the same insulin was given to another pair of animals, one fed, one starved, and in the case of the fed animal the hypoglycemia was only moderate in degree and there were no convulsions, whereas in the starved animal it was intense and convulsions developed in two hours. A still weaker dose had practically no effect on the well-fed animal, but a marked one on the starved one.

The Effects of Insulin on the Heart

The effects which follow the injection of insulin into a normal animal and from which we have drawn the conclusion, that sugar is removed from the blood to fill a vacuum for sugar in the tissues, would naturally lead us to enquire as to the effect of insulin on the rate of sugar consumption by the excised heart. Experiments bearing on this problem were undertaken by J. Hepburn and H. K. Latchford. The excised heart of the rabbit was perfused with oxygenated Locke's solution, containing glucose and the sugar determined in samples removed at regular intervals, the perfusion rate, the glycogen content of the heart, and the H-ion concentration being also observed. It was found that the rate of sugar consumption, without insulin, was on an average 0.8 mgm. per gram heart per hour, whereas it rose sometimes to 4.0 mgms. per heart gram per hour when this was added to the perfusion fluid. There is no doubt that insulin increases the rate of sugar consumption in the excised heart by three or four times. Why it does this, we do not know. The experiment really gives us little more information than that obtained by observing

the action of insulin on intact rabbits. It shows that the sugar has disappeared, that it has gone into the tissues, but it does not tell us whether it has become condensed into glycogen, or reduced to fat, or oxidized. It will take more work to be certain as to which of these processes, or perhaps all of them, may be responsible for the change. The importance of Hepburn and Latchford's experiment rests on the fact that it showed that the tissues absorbed more glucose when insulin was present.

The Assay of Insulin

The last part of the work, which I will bring to your notice, is that concerning the assay of insulin. As with every biological product, there is always great difficulty at the start in getting a method of assay. The original method suggested was that one unit of insulin is the amount required to lower the percentage of blood sugar in a normal rabbit of about 2 kg. weight to 0.045 within three or four hours.

Partly because of the confusion of using rabbits of different weights and partly because equal amounts of *insulin* may have varying effects on different rabbits, it is very difficult to devise a precise method of assay. There is a very little difference between a moderate dose and a large dose in the rate of fall in blood sugar during the first half hour, and even in the succeeding periods, the height of the blood sugar is largely dependent upon the glycogen stores in the animal's body. But, even if you standardize this condition, and make the glycogen stores uniform by starvation, the curves after the initial fall do not recover at the same rate. At no period, therefore, after giving insulin, can you be certain that the percentage of blood sugar is proportional to the dose of insulin. It is extremely difficult to arrive at an accurate method of assay.

Dr. G. S. Eadie and I have attempted to develop a method of assay by measuring the extent to which insulin can prevent the increase in blood sugar due either to the injection of fixed amounts of sugar or of epinephrin. The effect produced on normal rabbits by injecting 2 grams of glucose subcutaneously has been plotted in curves, and Dr. Eadie has shown by mathematical methods, the degree of variability to be expected in the curve. When insulin is given along with the sugar the rise in blood sugar is much less, and it lasts for a much shorter period of time. When the sugar is given ½ hour or 1¼ hours or 1½ hours after the insulin, the effect is more striking and it is most developed in about 1¼ to 1½ hours. After this the effect of insulin begins to pass off and 2 grams of glucose comes again to develop nearly the normal effect. These observations offer a method of assay which would consist in injecting 2 grams per kilo of glucose in 1¼ to 1½ hours after insulin, and then determining the extent of the rise in blood sugar.

In the method just described the insulin is matched against glucose introduced from without—exogenous glucose we might call it—but another method suggests itself, namely, whether the insulin could be matched against glucose derived from within—endogenous glucose. The best way to set free endogenous glucose is by epinephrin. If you inject epinephrin subcutaneously, you cause the glycogen to break down into glucose, and the possibility, therefore, exists that it might be possible to assay insulin in terms of epinephrin. This work has been done largely by Dr. Eadie who has found, by constructing a curve which shows the relationship between varying doses of insulin and increase in blood sugar following the injection of fixed amounts of epinephrin, that a fairly smooth curve is obtained, showing therefore, a mathematical relationship between the

two. Dr. Eadie has found a formula for this curve, the details of which I will not inflict upon you. Suffice it to say that the method is fairly accurate, except for the occasional aberrant results. Every effort of precise assay of insulin is difficult, because of these inexplicable aberrant results. It is like many other biological problems of similar nature; the aberrant result is a difficult one to deal with. We have, for example, observed in the laboratory, in one case, particularly, a rabbit that could stand four or five times the ordinary lethal dose of insulin with practically no effect, except the slight lowering of blood sugar. To this animal daily doses of insulin, sufficient to have killed several ordinary animals, were given for nearly two weeks, and at last when a lethal dose was given nothing could be found to explain the high resistance. It is possible that more accurate assays could be worked out by observing the blood sugar in completely depancreatised dogs. Partial pancreatectomy will, however, not furnish a useful animal for the purpose, since the endogenous production of insulin will necessarily be variable. For this reason it does not seem likely that patients will be useful for accurate assay since few if any cases are completely diabetic, that is, have no functional pancreatic tissue remaining.

Control of Insulin Secretion

In conclusion, let me say that there yet remains an entirely different part of our problem about which I have said nothing, namely, the control of the secretion of insulin from the Isles of Langerhans. What stimulates the production of insulin, and what damps it down according to the requirements of the body for this hormone? What is the mechanism of control? There are two possible mechanisms. Either it is controlled through other hormones, which may be the blood sugar, or

through the nervous system. In regard to control through the nervous system, I wish to state that Miss O'Brien and Mr. McCormick have recently obtained interesting though not quite conclusive evidence that the secretions of the Isles of Langerhans are controlled through the vagus, stimulation of which under certain conditions produces a lowering of the blood sugar. In this function the vagus would therefore appear to be antagonistic to the splanchnic.

BEAUMONT LECTURE III.

BY PROFESSOR F. G. BANTING

January 30, 1923

The Experimental Work upon Insulin. Its Use in Diabetes

I feel very inadequate in meeting the responsibility that falls upon me tonight in reporting the researches conducted by the various workers in the University of Toronto. Professor Macleod asked me to review the early experimental work carried out by Mr. Best and myself, and then to present the clinical aspects of the use of insulin in diabetes.

The idea from which this work originated presented itself from reading an article written by Moses Barron in *Surgery, Gynecology and Obstetrics,* in the November issue of 1920. In this article, which deals with the pathological changes of the pancreas following blockage of the pancreatic duct with gallstones, Barron points out the analogy between such degeneration and that which occurs following the experimental ligation of the pancreatic ducts in animals. Since ligation of the pancreatic ducts in animals causes degeneration of the acinous cells, but not of the islet cells, it seemed reasonable that by this procedure an extract containing the internal secretion might be obtained, which would be free from the deteriorating or toxic effects of the products of the acinous cells. The feasibility of this hypothesis being recognized by Professor Macleod, work was commenced

in the physiological laboratories of the University of Toronto, in association with Mr. C. H. Best.

E. L. Scott had attempted a similar procedure in 1912, but unfortunately he was unable to obtain degeneration of the acinous tissue. This is possibly due to the fact that when a ligature is tied very tightly there is necrosis of the tissue underlying the constriction, and by the time the ligature has necrosed through, there is sufficient fibrinous exudate deposited on the surface to reestablish the wall and lumen, thus failing to produce obstruction permanently. This may occur in ligation of the fallopian tubes. It is necessary then to ligate the pancreatic ducts just sufficiently tight to block the lumen, but not sufficiently tight to cause necrosis.

Work was commenced by ligating the pancreatic duct in a number of dogs. An interval of from seven to ten weeks was allowed for degeneration of the acinous tissue to occur. The dogs were then chloroformed and the degenerated remnant removed. Histological sections of this material showed replacement of the acinous cells by fibrous tissue. The degenerated remnant was cut into small pieces placed in ice-cold Ringer's solution, frozen, macerated and filtered. The filtrate thus obtained was tested by observing the effect of intravenous injection in a depancreatized dog. Depancreatized dogs, within twenty-four hours after the operation, develop a high blood sugar, possibly in the neighborhood of .250 per cent. On the second or third day the blood sugar is usually elevated to .300 per cent, and has frequently been observed as high as .450 per cent at the end of five or six days. The dogs' wounds, as a usual thing, fail to heal and the dogs seldom live longer than six or eight days, although they have been observed to live eighteen or twenty days. The urine contains persistently large amounts of sugar. Acetone bodies are present in but

slight amounts and are derived from the animal's own body fat, since in the absence of the pancreas, fats are not absorbed. For this reason, depancreated dogs are not suitable animals for the experimental work on acidosis and ketosis. As the fat is absorbed the animals become emaciated.

On July 31, 1921, the first degenerated gland extract was injected into a diabetic dog. This injection was followed by a reduction in blood sugar from .20 to .11 in one hour. The animal became sugar-free and was found to retain a larger amount of injected glucose than on a previous day when glucose alone had been given.

Only small quantities of extract were available for experimental purposes, owing to the difficulty in procuring dogs, and also to the length of time required for degeneration to occur. The next type of extract used was obtained from exhausted pancreas. This was done by the long continued injection of secretin into a normal dog, followed by the rapid removal of the pancreas before trypsinogen granules could be reformed. Active extracts were obtained by this means, but were unsatisfactory because it was not always possible to secure complete exhaustion of pancreatic juice, and the injection of these extracts was usually followed by toxic effects.

Lageusse found that a comparatively larger number of islet cells were present in the pancreas of the fetus and the newborn than in adult animals. It was first decided to extract the pancreas of newborn animals, but then it was thought that a more active extract might be obtained from the premature or aborted fetus. Finally the idea presented itself that since other glands of internal secretion contained their active principle as soon as they became differentiated in their embryological development, insulin would be present in the Islets of Langerhans. Furthermore, since pancreatic juice is not required by the fetus until delivery necessitates di-

gestion, pancreatic juices might not be present. It was later found that effort to demonstrate the presence of trypsin in the pancreas of the fetus of under four months' development had failed. An extract of fetal calf pancreas was made and injected subcutaneously into a diabetic dog. The injection was followed by a rapid fall in the percentage of sugar in the blood, unaccompanied by local or general reaction. The clinical condition of the diabetic dog was markedly improved, as evidenced by a more kindly healing of his wounds and the restoration of his normal friendly behavior. These effects of extracts of fetal calf pancreas thus indicated that the antidiabetic principle was in all probability universal in the animal kingdom. With the insulin thus obtained from the pancreas of fetal calves, an important step in the advance of the investigation was made, in that material was obtainable in more abundant quantities, and research could be pursued more rapidly.

Diabetic dogs rarely live longer than two weeks following total removal of the pancreas. In the endeavor to prove that extracts of the fetal pancreas contained the internal secretion, we tried to prolong the life of a depancreatized dog by artificial administration of the missing substance. Our first attempt at longevity resulted in keeping the depancreatized dog alive for nineteen days. This dog died from lack of extract. Our second attempt resulted in the dog's living twenty-one days. At this time we had prepared a new extract from fetal calf pancreas. About two hours after the injection of a 5 c.c. dose, the dog developed a peculiar anaphylactic-like reaction, which was characterized by stupor, drowsiness and coma, and finally periods of convulsive twitching and death. This reaction was not recognized at this time as due to hypoglycemia from an overdose. Our third attempt at longevity resulted in keeping a totally depancreatized dog alive for seventy days. At

the end of this time, although extremely weak and emaciated, she was able to run about the floor, scratch fleas on the back of her neck, and was in fairly good condition. The dog was chloroformed, and an autopsy immediately performed by Dr. Robinson, pathologist to the Toronto General Hospital. A careful search was made for any remnant of pancreatic tissue, but none was found, except a small plaque 2 mm. in diameter and 1 mm. in thickness, between the mucous and the muscular layers of the duodenum. On serial sectioning of this plaque no islet tissue could be demonstrated.

The main object of the investigation at this time, (November, 1921) was to obtain a means of chemical extraction of the active principle which would destroy, precipitate, or leave undissolved the deteriorating or toxic elements of the acinous cells. With this object in view, various percentages of ethyl-alcohol were used. It was found that the active principle was soluble in all percentages up to 70, but insoluble in 95 per cent.

The procedure of making alcohol extract was as follows: Equal volumes of 95 per cent alcohol slightly acidified by the addition of hydrochloric acid, (0.2 per cent), and macerated fetal pancreas were mixed. The mixture was filtered and clear straw colored filtrate was obtained. This was evaporated to almost dryness in a warm current of air. The resin-like residue was redissolved in distilled water and its antidiabetic principle tested on depancreatized dogs. By applying this procedure to whole adult beef pancreas, an active extract was obtained. A purer and more potent product resulted from distillation of the alcohol in vacuum at low temperature. This form of extract was tested on three patients. In the first patient to whom it was given there was a 30 per cent reduction in blood sugar and a marked reduction in the amount of sugar excreted in the urine.

When absolute alcohol was added to 70 per cent ex-

tract, a fine whitish precipitate of the active principle, to which the name insulin was given, was formed. This precipitate disappeared on adding water. About this time, January, 1922, Dr. Collip became associated in the work, and was able to fractionally precipitate the active principle, and by this procedure was able to obtain a more refined extract of whole gland.

Clinical Investigations With Insulin

At this time the clinical investigation was taken up by Doctors Campbell and Fletcher of the medical staff of the University of Toronto, under the directorship of Professor Duncan Graham. The large scale production of insulin was made possible by facilities provided by the Connaught Laboratories, under the direction of Doctors Defries and Fitzgerald, and the physiological problems were allotted by Prof. Macleod to pairs of workers. The results of these were reported by Professor Macleod in the foregoing lectures. In the first clinical cases it was found that the percentage of sugar in the blood could be reduced to normal, the urine could be rendered sugar-free, that acetone bodies disappeared, and that patients gave evidences of marked clinical improvement. These results substantiated the findings obtained in experimental animals. Unfortunately, difficulties were encountered in changing from the small to the large scale production, and we were forced to abandon the clinical investigation for the course of about three months. For the establishment of large scale production we are indebted principally to Mr. Best.

In May, 1922, with Dr. Gilchrist, the clinical investigation was commenced at the Christie Street Hospital for Returned Soldiers. In August a diabetic clinic was established at the Toronto General Hospital by Prof. Graham, and later the work extended to the Hospital

for Sick Children, in association with Doctor Gladys Boyd. The contributions from these sources will be discussed as a whole.

A patient, on admission to the hospital, usually remains on the same diet as previous to admission, for about twenty-four hours, in order that the physician may gain some idea of the severity of the case. He is then placed upon a basal requirement diet, which is estimated by the use of DuBois chart and Aub-DuBois table, the food values of which are calculated from the Wilder, Woodyatt, (or slightly modified) ratio. The patient remains on this diet at least a week, unless severe acidosis supervenes. *It is necessary before commencing insulin treatment to ascertain the patient's tolerance for glucose.* This is done by subtracting the average daily excretion from the daily intake. During this trial period, blood sugars are taken before breakfast, and three hours after, in order to determine the effect of fasting and of food on the blood sugar level. Daily estimations are made of the amount of sugar and acetone excreted in the urine. After a careful history has been taken, the patient is given a complete physical examination. Special attention is directed to possible foci of infection; the teeth, tonsils, accessory sinuses, chest and digestive system are examined clinically. If any source of septic absorption is located, it is appropriately treated.

If the patient becomes sugar-free on this basal requirement diet, the diet is raised, and if it is found that he is able to metabolize six or seven hundred calories over and above his basal caloric requirements, his case is not considered sufficiently severe for insulin treatment at the present time. It will be found that on this balanced diet which contains sufficient protein to maintain nitrogenous equilibrium, and in which the ratio between carbohydrate and fat is such as to prevent ketosis, that

the daily excretion of sugar becomes fairly constant. Patients who constantly excrete sugar on a diet of less than five hundred calories over and above their basal requirement are placed upon insulin treatment. Insulin treatment consists in the artificial administration of the internal secretion of the pancreas in sufficient amounts to compensate for the deficiency in production of the patient's own pancreas.

Dosage of Insulin

The dose of insulin is calculated not only from the amount of sugar excreted in the urine, but also from the extent of hyperglycemia. *A unit of insulin is the amount of active principle required to reduce the blood sugar of a normal one kilogram rabbit, which has been starved eighteen hours, from its normal level of approximately .123 per cent to .045 per cent.* Although there is at present a slight variation in the strength of the unit, it is equivalent in a moderately severe diabetic to about 2.5 grams of glucose, or in other words, one unit of insulin causes about 2.5 grams of glucose to be burned. In milder cases the utilization per unit is more than this amount, while in cases complicated by infection with an elevation of temperature, the utilization per unit is less. There is also a variation in the individual response on the part of the patients. For example, two patients in the same ward were each given twenty units of insulin. Their blood sugars were .300 per cent and .320 per cent, respectively. They were on the same diet and excreted fifteen to twenty grams of glucose per day, and thirty to forty grams of glucose per day, respectively. The patient with the high blood sugar and who was excreting the larger amount of sugar was thrown into a severe hypoglycemic reaction, about two hours after the dose, while the other patient

did not become sugar-free. It is therefore advisable in commencing insulin treatment to give small initial doses and gradually increase until the blood sugar is reduced to the normal level and the patient is rendered sugar-free.

Administration of Insulin

Insulin is administered from twenty minutes to a half hour before meals. In severe cases it is sometimes necessary to give three doses per day, but in the usual case two doses per day, one before breakfast and the second before the evening meal, prove sufficient. Milder cases may be adequately controlled on one dose per day, given before breakfast. The hypoglycemic property of insulin counteracts the hyperglycemia, otherwise produced by the meal, in such a way that the patient's blood sugar remains below the kidney threshold, and the patient remains sugar-free. It is possible to maintain even the most severe diabetic sugar-free by balancing the artificial administration of internal secretion of the pancreas against the diet which is not accounted for by the patient's own secretion. When this balance is reached, or in other words when the patient is sugar-free, the diet is raised three hundred calories every second or third day, until he is receiving about twice his basal requirement. This applies only to the emaciated diabetic, as the overweight diabetics are best treated by dietetic treatment.

It seems desirable that diabetics be maintained sugar-free, in order that their islet cells may have a chance to recover. This is particularly desirable in young patients. In diabetics who have had a persistently high blood sugar, and have daily excreted large amounts in the urine, a definite increase in tolerance is observed after they have been maintained sugar-free. These results are similar to those previously obtained by dietetic

treatment. There is no doubt that the presence of an increased percentage of sugar in the blood stimulates the islet cells to produce insulin, consequently it seems reasonable to believe that the long continued relief of over-strain on the islet cells may permit of improvement in their function, and result in permanent benefit. This is further substantiated by the fact that patients require progressively smaller dosage to maintain them sugar-free on a constant diet.

Overdosage and Symptoms

When an overdose of insulin is given, the blood sugar is lowered below the normal level, and this results in a symptom complex, which we call hypoglycemic reaction. This is characterized by a feeling of uneasiness, perspiration, flushing or pallor, increased pulse rate, tremulousness, although there is no visible tremor, a feeling of impending danger, incoordination of speech and movements, stupor, and coma, and finally convulsive seizures. The level at which these symptoms are noted is usually about .060 per cent, but if the blood sugar has been rapidly reduced from a high level, of for example, .400 per cent, the reaction may commence as high as .150 per cent. The lowest percentage of sugar in the blood observed during hypoglycemic reaction is .032 per cent. The subjective sensations are pathognomonic, and once experienced by a patient are recognized very early, consequently we endeavor to produce mild hypoglycemia in all patients while under observation in the hospital, so that they will be the better prepared, should such an accident occur after their discharge. Fortunately the treatment of hypoglycemia is easily carried out. The object is to increase the percentage of sugar in the blood. This is done by the oral administration of glucose, in the form of glucose candy, orange juice

or sugar. If the patient is in coma, due to the hypoglycemia, ten to fifteen minums of epinephrin subcutaneously will cause enough glycogen to be released from the liver to raise the patient's blood sugar sufficient for him to regain consciousness. Glucose should then be administered to prevent further reaction.

Acidosis and Coma

A diabetic who may still have a good tolerance for carbohydrate, at some time may develop acidosis by the over-indulgence in food, or by toxemia or infection. As long as the excretion of ketone bodies keeps pace with the production, simple acidosis prevails, but when the production of ketone bodies exceeds the excretion, they accumulate in the system, producing air hunger, drowsiness, and finally coma. Insulin is specific in the treatment of diabetic coma. Of twelve patients treated, eight have recovered. The first case died because we were unable to obtain insulin; the second case died of pneumonia; the third died from cardiac failure, and the fourth died of septic gangrene. This patient would not cooperate, in that she refused to allow surgical interference. The three later cases were rendered sugar- and actone-free previous to death. One case may be reported in more detail, that of a child twelve years of age, in whom glycosuria was first noted in April, 1922, following an attack of follicular tonsillitis. She was placed on a diet, which was liberal because her tolerance was high. However, the child did not adhere to it, and by August she was again showing sugar in the urine and was losing weight rapidly. Increased thirst and appetite again became prominent symptoms. On the eleventh of September the child ate a large quantity of grapes and a number of olives. Following this she suffered from stomach ache, nausea and vomiting, then

developed drowsiness and finally coma. She was admitted to the Hospital for Sick Children on September 13th, at three P. M. On admission the blood sugar was .500 per cent, the urine contained 7 per cent sugar and gave a four plus Rothera test for acetone. Forty units of insulin were administered subcutaneously at three-thirty P. M. At five o'clock the blood sugar was found to be .200 per cent. By seven o'clock it was further reduced to .100 per cent. The child was recovering from coma. At seven-thirty, 17.5 grams of glucose were administered intravenously, but despite this glucose, the blood sugar had fallen to .080 per cent at nine o'clock. On the morning of the fourteenth, the whole clinical picture was changed. The acetone in the urine had dropped from four plus Rothera test to a faint trace. The blood sugar which on admission was .500 per cent was now .08 per cent, and the urine was sugar-free. The child became very restless, tossing about her bed, and while previously she had been unable to articulate, she was now talking in a wild delirium. The temperature rose in the course of a few hours from 101° F. to 105° F. The pulse became very rapid. Although the chest was repeatedly examined, no signs of pneumonia could be found. The clinical picture was clearly one of a severe toxemia. The temperature continued to rise and reached 106.2 degrees, the pulse became more rapid, (140). On the previous day an enema had been given but with slight result. At noon, September fourteenth, a series of enemata, every three hours, were commenced, soda bicarbonate was administered per rectum, and each specimen of urine was tested to detect alkalosis. Insulin and glucose were repeatedly administered. At six o'clock, the morning of September 15th, the child passed a very large amount of putrefying foul-smelling fecal matter. The temperature and pulse soon fell to normal and the clinical condition of the child rapidly improved.

Six days after admission the child was placed on a weighed and calculated diet and rendered sugar-free with insulin. The diet was gradually raised from 420 calories to 1400 calories during the next three weeks. Decreasing doses of insulin were required. On October 13th, insulin treatment was discontinued and the diet reduced to 420 calories. This diet was then gradually raised to 1470 calories, when the child was discharged from hospital, November 13th. No sugar appeared in her urine after the sixth day in the hospital. This case is an example of coma precipitated by toxemia.

Insulin in Complications of Diabetes Mellitus

Insulin favorably influences many of the complications of diabetes mellitus. Boils and carbuncles are a frequent occurrence, and are a serious menace to the general health of the patient. It would appear that the severity of these is due in part to hyperglycemia, because when the blood sugar is rendered normal, and glucose is burned by the body, infections and infected wounds tend to heal rapidly. Tuberculosis in diabetics usually progresses rapidly and ends fatally, and heretofore was particularly difficult to treat because it was impossible to feed the patient an adequate amount on account of the diabetic condition. Two cases of pulmonary tuberculosis with low carbohydrate tolerance have been treated and are progressing favorably on the increased diet, obtainable by insulin treatment. Patients with elevated temperature and infection such as influenza or tonsillitis, require increased doses of insulin to maintain them sugar-free on a constant diet. Diabetic gangrene is primarily due to damaged capillary circulation with the resultant improper nourishment of the tissues. Insulin does not improve the circulation, but may favorably influence the superimposed infection. The

great value of insulin in this type of case is that it permits operative interference because the patient may be rendered acetone-free and sugar-free by its use.

Symptoms of diabetes are produced, primarily by the failure on the part of the body to burn adequate amounts of carbohydrate. This causes glucose to accumulate in the blood, giving rise to thirst, and since large amounts of fluid are taken into the body, large amounts are excreted. In children, owing to the large excretion of water, the frequent emptying of the bladder at night is a pronounced symptom. Since glucose is not metabolized, the tissue cells are not properly nourished, giving rise to constant hunger, and despite the ingestion of large amounts of food there is progressive loss in weight. The unfavorable prognosis and the limited diet, in the presence of constant hunger frequently produce a mental condition characterized by pessimism, irritability and even melancholia. Insulin enables the body to burn carbohydrate, therefore all of these symptoms are relieved.

I wish to express my sincere thanks to all those associated in the work, and to express my appreciation of the many kindnesses and much helpful encouragement from friends and patients.